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A SYSTEM OF
BACTERIOLOGY
IN RELATION TO MEDICINE

VOLUME VII

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For this Volume Professor R. T. Hewlett and Dr. F. C. Minett have assisted in the editorship with regard to particular sections.

A
SYSTEM OF BACTERIOLOGY IN RELATION TO MEDICINE

VOLUME VII

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CHAPTER I. VIRUSES AND VIRUS DISEASES : INTRODUCTORY SURVEY.

By W. E. Gye (National Institute for Medical Research) and J. C. G. Ledingham (Lister Institute, London).

Introduction.

The possibility of the existence of excessively small invisible micro-organisms was first suggested by Pasteur in his researches on the ætiology of hydrophobia, but the earliest evidence proving the existence of such invisible contagia was obtained by Iwanowsky, who found that mosaic disease of the tobacco plant could be transmitted by extracts of the diseased leaf which had been filtered through a porcelain filter. This work was independently confirmed by Beijerinck. Loeffler and Frosch in 1892 discovered that when vesicle fluid of foot-and-mouth disease is largely diluted and then filtered through porcelain candles which hold back all visible bacteria the filtrate retains its infective power almost unimpaired. Microscopic examination of the filtrate, either under the dark field or by means of stained films, failed to reveal a micro-organism ; cultures on artificial media invariably failed. These primary observations, which have been everywhere confirmed, prove that an agent exists in vesicle fluid which is so small that it passes readily through the finest filter-candle, is invisible under the best microscope, and is able to initiate in a sensitive animal the disease process. It causes no other disease and is thus the specific cause of foot-and-mouth disease. The agent multiplies in the body of an affected animal, but has not so far been cultivated in the test-tube.

The pioneer work of Iwanowsky, Beijerinck, and Loeffler and Frosch thus established the existence of a new class of infective agent. Evidence of varying degrees of completeness has now been obtained of a hundred and more diseases of plants and animals caused by such agents. Many names have been given to the agents but none is entirely satisfactory. They have been designated as invisible microbes, ultramicroscopic viruses, inframicrobes, microplasms, strongyloplasms, chlamydozoa and filterable viruses. Each name is designed to indicate some special peculiarity, usually one dependent upon size, such as invisibility under the microscope or easy filterability, or some supposed biological peculiarity. The name filterable virus has become the most commonly employed term, but since some of the genuine examples of the class are not filterable and some other microbes are filterable and are not viruses (e.g. *Leptospira*), it would be preferable to omit the word filterable and use the term virus as applicable only to this class of organism.

At the outset of this introductory survey it may be well to state that the study of viruses affecting animals and plants has in our view revealed but few peculiarities other than dimensional which mark them off unequivocally from visible microbes. Of these peculiarities there is perhaps only one on which particular emphasis may be laid, and that is the association with a number of virus diseases of peculiar inclusion bodies in cells of the lesion. Should these inclusions, however, be ultimately shown to represent masses of elementary virus bodies which have invaded noble cells, and there would appear to be some good evidence of this in the case of the Bollinger bodies of fowl-pox, the analogy with pictures of included lepra bacilli, tubercle bacilli, gonococci, meningococci and *B. tularense*, would be complete.

The failure in all but pleuro-pneumonia to cultivate viruses *in vitro* apart from living tissue—and this failure applies to bacteriophage as well—has stimulated some minds to venture the hypothesis that viruses are not living entities but catalysts or ferments capable after entering the susceptible cell of so affecting its metabolism that more catalyst of the same kind is produced, which then proceeds to 'infect' more widely. This theory does not seem to be in accord with what we know of the action of ordinary ferments and catalysts, which exhibit no powers of reproduction when in contact with specific substrates. The onus of proof, therefore, is with those who have put forward these speculations, and so far no such proof is forthcoming. The matter is more fully discussed below. At present we must be content to review those features of viruses which serve to strengthen the standpoint that in all material particulars apart from size, which naturally presents its own problems, viruses live and move and have their being much as the visible pathogenic microbes. Even size may yet prove a not insuperable barrier to instructed vision, at any rate in the case of the less easily filterable sorts.

Are Viruses Living Particulate Organisms Capable of Cultivation?

Discussion of this question is governed by the basic fact that viruses multiply illimitably in the tissues of affected plants and animals. The simplest theory of the nature of viruses is that they are living organisms, and on this view the basic fact of multiplication is explained naturally: the proof of multiplication of viruses is, indeed, demanded as an integral part of the theory. The viruses, according to this theory, originate extrinsically to the cells of an affected animal.

There is no clearly formulated alternative explanation of the nature of viruses. The suggestion put forward by Sanfelice in 1914 that a virus is a non-living poison elaborated by affected cells and possessing the power to attack new cells, provoking in them the formation of a further quantity of the poison, forms the basis of most rival theories. It will be observed that the undoubted fact of multiplication of viruses is thus thrown back upon the living cell. By postulating an intrinsic origin for viruses the difficulties presented by multiplication are more or less surmounted.

Explanations of this kind are unsatisfactory, however, inasmuch as they are both unnecessarily complex and fail to explain some of the well-recognized properties of viruses. In recent years, nevertheless, a tendency to cast doubt upon the probable validity of the view that viruses are living organisms has appeared, and demands attention. The probable reasons for this tendency to favour complex explanations of the established facts are three in number: (1) the difficulty of cultivating viruses in the absence of living tissues; (2) the difficulties which have been added to the subject by the discovery of the bacteriophage; (3) the apparent impossibility of explaining the acknowledged facts of fowl tumours, particularly in relation to mammalian new growths.

Cultivation. With regard to the first of these difficulties it has so far been found impossible to cultivate typical viruses in the absence of living tissue. Vaccinia virus has been proved to multiply in a medium consisting of Tyrode solution and blood-serum to which a fragment of fowl kidney is added (Maitland and Maitland, 1928). When the fragment of kidney is first subjected to freezing and thawing, a process which kills the cells, and then added to the medium it is found that multiplication of vaccinia virus does not take place (Harde, 1916; Rivers, Haagen and Muckenfuss, 1929[2]). The kidney cells must therefore be alive if the virus is to multiply. This dependence of the virus upon living cells for its multiplication outside the body as well as in the body, is proved for a number of viruses and for bacteriophage, and may be regarded as lending support to the hypothesis that the living cell is stimulated by virus to manufacture more virus, the assumption being that the virus is a product of the cell. These deductions are, as will be shown later, difficult to reconcile with other well-established facts relating to the natural transmission of some virus diseases, but they ought also to be contrasted with the fact that the organism of bovine pleuro-pneumonia is readily cultivable on horse-serum-agar. Many writers now tend to regard the organism of pleuro-pneumonia as a microbe proper and as having no relation to viruses. The grounds for this opinion are never clearly stated. The organism is very small—$0 \cdot 15\mu$ approximately, and therefore unresolvable under the best microscopes in common use—and is easily filterable through candles, such as the Chamberland L2 or Mandler. In order to demonstrate the organism microscopically, it is necessary either to stain colony preparations heavily with Giemsa dyes, in which case the organisms are artificially enlarged, or to use Barnard's dark field apparatus in which the numerical aperture is $1 \cdot 2$. Even with this microscope the particulate organisms are not clearly resolved, the limit of achievement being a more or less satisfactory separation. For clear resolution it is necessary to use ultraviolet light. The organism is unlike the class of anaerobic cocci and bacilli—e.g. *B. pneumosintes* of Olitski and Gates—which, when first isolated, are small and may pass through filter-candles, but which with artificial cultivation become larger, non-filterable and easily recognizable. It retains its fixed characters as to size and the peculiarities of subdivision discovered by

Barnard. There are, therefore, no satisfactory grounds for classifying the organism among ordinary bacteria. It is more in accordance with known facts to regard it as a large and cultivable virus. If this viewpoint be taken, the failure to cultivate any other virus may be looked upon as due to technical difficulties which may be overcome as knowledge increases. Alternatively it is possible that the majority of viruses are obligatory cell parasites, multiplication depending upon the provision of conditions, at present undefined, which exist within living cells.

The difficulties introduced into the subject by the facts brought to light concerning bacteriophage are not such as to throw the balance entirely in one direction. Although the hypothesis that the bacteriophage is a transmissible property of cells is strongly advocated by Bordet and many other observers, the view that the agent causing dissolution of bacteria is a living organism has its supporters in d'Herelle and others. The general properties exhibited by bacteriophage are those usually associated with living organisms—multiplication, adaptability, antigenic properties, sensitiveness to antiseptics and so on—but the real difficulties are, first, the obligatory association of bacteriophage with a living organism, and, secondly, the more or less strict specificity possessed by each example of the bacteriophage. This latter difficulty is exemplified most strikingly by the tumour viruses. There are a dozen fowl tumours caused by filterable agents which are endowed with the property of multiplication in association with the appropriate fowl cell. These tumours differ widely in structure and the causative agent of each one is specific both to fowls and to cell-type of tumour. These viruses thus possess the properties usually regarded as characteristic of living micro-organisms and properties which are most easily explained as being characteristic of animal cells. The difficulties which confront the opinion that the filterable agents of tumours are living organisms are complicated by opinions as to the real nature of new growths derived from the studies of morbid anatomists. The difficulties are admitted by all students, but it is not permissible to transfer them to the problem of the animate or inanimate nature of the whole class of filterable viruses.

The capacity to adapt themselves to a changed environment is highly characteristic of living things and is shown strikingly by some viruses. The first example of this is found in the adaptation of smallpox virus in calves. When smallpox virus has been passed through a sufficient number of calves in succession, or even through rabbits only, it becomes 'vaccinia' and does not, even after repeated passages in man, return to its original condition. Similar phenomena are observed in the adaptation of herpes virus to brain tissue, of contagious epithelioma of fowls to pigeons, and of the mosaic disease of tobacco to cucumbers. It is difficult to reconcile such facts with any conception of viruses as inanimate substances.

The highly contagious virus diseases, such as foot-and-mouth disease and dog distemper, provide epidemiological evidence which appears to rule out of account the theories which explain viruses as products of diseased cells. Dogs and ferrets are both very susceptible to distemper, the disease spreading with the greatest ease from one species to the other. It is not essential for direct contact between the affected animal, a dog, for example, and a healthy animal—ferret—to take place; the infection spreads to a limited extent by air (Dunkin and Laidlaw, 1926). If the virus of distemper be regarded as a by-product of the cells of dogs, it is as inconceivable that ferrets should be so easily infected as it is that dog and ferret cells should have in common a peculiar property of being able to manufacture the same curious infective by-product under a given impulse. In the virus diseases which are spread by insects, e.g. yellow fever, dengue fever and certain mosaic diseases of plants, the difficulty is even greater. Here the virus is taken into the body of a mosquito or an aphis, there to sojourn a variable length of time before infection can be passed on again. The conception that a part of a cell of a man suffering from yellow fever may remain for days in the body of a mosquito, probably undergoing changes meanwhile, and then be transferred to the body of a healthy man, there to stimulate again the production of similar by-products, is too difficult to be readily accepted.

It may be worth while, however, to consider more particularly the implications of a view such as that of Boycott (1929), who decides that it is impossible at the present moment to find any criterion that we can apply to enable us to distinguish between two possibilities, viz. (1) that a virus behaves like a bacillus and (2) that it behaves like a growth-promoting substance. An extract of embryonic tissue stimulates the growth of connective tissue *in vitro* with the result that at the conclusion of the experiment, if the resultant growth is allowed to autolyse, more growth-promoting substance is available. The process of multiplication of growth-promoting substance here is not, in his view, dissimilar from what one gets with a virus, the multiplication of which is 'always associated with live cells and with the growth of live cells'. In our opinion Boycott does not take into consideration what has been learnt from the histopathology of the virus lesion. When the virus of vaccinia, for example, is introduced into the dermis, certain cells of the reticulo-endothelial system are observed to break up, and polynuclear cells that enter the lesion in great numbers are also destroyed. In the karyorrhectic focus thus developed more virus is demonstrable by animal inoculation than went in. The growth of reticulo-endothelial cells which subsequently takes place is a secondary defence phenomenon entirely analogous with what takes place in the healing of an erysipelas lesion. It is impossible here to demonstrate any analogy between a virus and a growth-promoting substance which acts presumably only as an accessory food factor. It should be emphasized also that the growth of vaccinia virus which has been realized by the Maitlands in the presence of autolysing tissue may not be dissimilar from the 'growth' observed *in vivo* in a karyorrhectic focus of the dermis. Evidence of growth of virus *in vitro* in contact with living tissue has been obtained although the tissue has shown no signs of growth. It is probable, therefore, that the food-stuffs required for the growth of virus *in vitro* may be some particular degradation products of cell protein yet to be identified and tested.

If viruses are living objects they must be particulate. Have we any evidence in support of their particulate nature? Centrifugalization has been employed as a method of obtaining an answer. D'Herelle has shown that bacteriophage can be shifted from the upper layers of a fluid towards the lower layers by spinning the fluid for a long period of time.

Other viruses have been found to be similarly affected by gravity. But these observations are largely nullified by the fact that viruses attach themselves readily to protein particles which carry them down when spun in the centrifuge. When protein particles are removed by a preliminary filtration through a close filter-candle, and the filtrate, thus freed of all particles visible under the dark field, is spun, the rate of spinning required to diminish the infectivity of the upper layer requires to be greatly augmented, from, for example, 4,000 revolutions to 9,000 revolutions a minute. The speed required to shift a free virus thus comes into the region where complex colloidal aggregates are affected. We are therefore left without any definite answer to the question, which is probably insoluble by such a method.

The fact that in the electric field, viruses like bacteria are negatively charged and migrate to the anode is, of course, no argument in support of their particulate nature, but the correspondence in this respect is at least notable.

The Size of Virus Particles.

At present there is no reliable information as to the size of the viruses. The usual method of attempting to estimate size is to compare the filterability of a given virus through a particular collodion filter with the filterability of colloidal particles of known size through the same filter. Various colloidal particles of different sizes are used, but since it is difficult to determine definitely the size of the colloidal particles it is obviously more difficult to give value to the estimated sizes of viruses. The bacteriophage is a favourite object of study in these investigations. D'Herelle (1925) estimates the diameter of the phage as 20 to $30\mu\mu$, whilst Bechhold and Villa (1926) calculate its size as between 35 and $200\mu\mu$. Zinsser and Tang (1927) conclude from their studies that the bacteriophage, herpes virus and the virus of the Rous sarcoma No. 1 are between 20 and $100\mu\mu$. Elford (1929), with the most refined collodion technique, estimates that the particles of the organism of bovine pleuro-pneumonia are $0{\cdot}15\mu$, an estimation which agrees closely with Barnard's measurement of the same organism by ultraviolet microscopy.

Problems of Filtration.

When the morbid material containing the virus is a fluid, as, for example, vesicle fluid in foot-and-mouth disease or blood or cerebrospinal fluid in canine distemper, the fluid is diluted and cleared if necessary by spinning and then filtered through a candle. Where the virus is attached to a solid tissue as in fowl sarcomas the tissue is disintegrated by pounding with sand, the mass diluted with the extracting fluid, cleaned of gross particles by centrifugalization, and the clear fluid then passed through a filter. Sometimes it is found advantageous before candle filtration to remove coarse particles of tissue debris by passing the turbid muddy extract of broken-up tissue through a filter made up of alternate layers of paper-pulp and sand. A useful method of obtaining an extract of an infected tissue consists in incubating pieces of the tissue in broth or serum-broth or in saline. The infective virus may diffuse out into the fluid under suitable circumstances and the diffusate be then filtered. This is a satisfactory method of obtaining herpes virus from brain tissue. Filtration through candles is an uncertain process and must be checked by including some small organism—e.g. *B. prodigiosus*—in the fluid to be filtered. The filtrate must be shown to be free of the test organism, and to contain the virus under examination, the latter being proved by animal test. The results of filtration are affected by a number of conditions: by the duration of filtration and by the pressure employed to force fluid through the candle, by the viscosity, the temperature and acid or alkaline reaction of the fluid filtered, by the adsorption of the virus to protein aggregates and by possibly unknown conditions. The size of the virus does not necessarily play a predominant part in deciding the result of filtration. The organism of pleuro-pneumonia, regarded by some writers as an ordinary microbe, passes readily through a Chamberland L2 candle. This candle constantly prevents the passage of vaccine virus. Nevertheless the organism of pleuro-pneumonia is visible by dark-ground microscopy and is probably a larger organism than vaccinia virus. It follows from this that filtration through candles is not of theoretical importance in the investigation of viruses, although it is always desirable where possible to effect such filtration in ætiological investigations. We are uncertain, for example, whether certain plant viruses that are transmissible only by grafting or budding are filterable or not. In any case a plant juice containing the virus has not yet been obtained.

The numerous disadvantages possessed by porcelain and other similar candles have led to the use of collodion filters in efforts to reduce filtration to a process in which size of particle largely determines the passage or non-passage of the particle through the filter. The most suitable form of collodion filter is that introduced by Bechhold. It is made by soaking filter-papers in a solution of gun-cotton in glacial acetic acid, the acid being subsequently removed by washing in distilled water. This method of collodion filtration has been exploited by Barnard and Elford and by Zinsser and Tang. It has been shown by the former observers that by using one stock of gun-cotton in order to avoid the variations in physical properties which different samples of chemically similar gun-cotton exhibit, and by choosing a satisfactory type of filter-paper for impregnation, the porosity of the filter may be varied at will by altering the concentration of the collodion. It is thus possible to prepare filters which separate particles of, for example, $0 \cdot 1\mu$ from a mixture containing particles of $0 \cdot 2\mu$. By the development of such methods it is probable that viruses of different sizes may be separated from one another. These filters, however, demand special experience and skill in their preparation and cannot at present replace candles for ordinary purposes.

Action of Physical and Chemical Agents on Viruses.

Most of the viruses are relatively resistant to the action of 50 per cent. glycerol, differing in this respect from most visible microbes. The difference is not absolute, however; some viruses—e.g. virus III—some tumour viruses, &c., become inert in six weeks, whilst many bacteria remain viable for a longer period. An absolute difference between viruses and visible bacteria could not be expected. Likewise, differences between individual members of either class are found. In general it may be said that the wide range of difference between members of the class of visible bacteria towards the action of physical agents—ultraviolet light, heat and cold—and chemical agents—antiseptics—are met with in the viruses. Like ordinary bacteria all viruses are destroyed at high temperature. Some are destroyed at relatively low temperatures, e.g. 45 to 55° C., in a short time, others resist temperatures of 70° C. for 15 minutes. In so far as the rate of sterilization of viruses by heat has been studied, it has been found that the laws governing the rate of action are the same for viruses as for ordinary bacteria.

Some viruses remain active in the dried state for years. The fowl tumour, Rockefeller Institute Series, No. 1, when dried rapidly and kept *in vacuo* over phosphorus pentoxide, is still active after six years. Other viruses, including some of the tumour viruses, lose their activity after a few weeks or months. Some dried viruses may be extracted with dry ether, chloroform, xylene, or acetone, and still remain active. Most viruses, however, are inactivated by these chemicals in the wet state. Phenol in 1 to 2 per cent. strength destroys many of the viruses, but 3 per cent. solution is insufficient to inactivate the virus of African horse sickness.

There are no common properties of the virus class of contagia which suffice to enable a distinction to be drawn between bacteria and viruses.

Epidemiological Features of Virus Diseases.

It cannot be said that in the matter of epidemiological features, virus diseases present peculiarities that are not met with in frank bacterial infections. While some, like virulent smallpox, are extremely contagious, others, like the virus of poliomyelitis, which enters the body most probably by the same route as smallpox, are of low infectivity. Like the meningococcus, the virus of poliomyelitis would appear in times of epidemics to be fairly widely distributed among carriers, while cases are exceptional. Dog distemper may be placed alongside smallpox as a highly contagious disease where any very immediate contact is unnecessary for infection, and, indeed, in experimental work on this disease of dogs, it has been found essential to breed the animals required for experiment, in view of the difficulties of excluding infection by ordinary quarantine methods. Under laboratory conditions it is not always possible to ensure natural processes of infection and transmission even when the animals are susceptible in nature to the disease under investigation. Though fowl-pox, for example, readily spreads in an infected poultry-yard, it is rare for infection to take place from an infected hen to a healthy fowl placed in contact with it, and this though the disease is so readily transmitted experimentally by insertion of virus-containing material into some skin abrasion. It is possible that, even in nature, biting insects may play a role in transmitting diseases of this type. Experimentally at least, it has been shown (Kligler, Muckenfuss and Rivers, 1929; Kligler and Ashner, 1929) that fowl-pox can be conveyed in this way.

Infectious myxomatosis of rabbits also does not require direct contact between infected animal and healthy. Vaccinia can ordinarily be passed only by transferring the virus to an abraded skin or mucous surface, but it is now claimed that highly potent strains of vaccinia virus such as neurovaccine or neurotesticular vaccine may by some unobserved route (probably respiratory) infect healthy rabbits when such are kept in the vicinity of experimentally infected animals. Such strains of vaccinia would thus behave in the rabbit like smallpox virus in man.

Of those viruses requiring direct insertion into an abraded skin or mucous surface, rabies may be cited, though it has been held that actual abrasion may at times not be necessary, e.g. where the virus has fallen on some highly absorbent surface such as the conjunctiva. The recent claim by Cowdry (1927) that the virus associated with the Japanese epidemic encephalitis is identical with that of rabies, raises a new problem in rabies epidemiology.

Infection by the intestinal route may be illustrated by such diseases as hog cholera, foot-and-mouth disease, sacbrood and polyhedral disease of insects. With regard to foot-and-mouth disease, however, the actual site of entrance of the virus may be in the upper nasopharyngeal area through some mucosal abrasion, and, indeed, in many cases it is as difficult to decide the exact point of entrance of virus into the general circulation as, e.g., in enteric fever.

The transference of infection from sick to healthy, by means of insect vectors, including lice, ticks, mosquitoes, sandflies, mites and plant aphides, is illustrated by such diseases as typhus and trench fever (lice), rocky mountain fever and heartwater (ticks), tsutsugamushi disease (larval mites), pappataci (sandflies), dengue (mosquitoes) and the plant mosaics (aphides).

The specificity of the insect vector in diseases like typhus fever, trench fever, dengue fever, is believed to be almost absolute. In the mosaics, however, that are spread by aphides, several different species may be responsible or only one type may be potent. So far as we know, the virus has no action on the tissues of these aphides, though it is significant that in some plant viruses there is a period after the aphis has fed on the plants during which it is incapable of conveying infection to a healthy plant. Though in the majority of virus diseases of plants an insect vector is responsible for conveying infection, in a minority, the virus cannot be mechanically transmitted in this way but only by some procedure such as grafting or budding (e.g. abutilon mosaic, aster yellows, peach yellows). That the infection produced by the plant aphis is not a mere mechanical transference comparable with that effected by the needle is shown by the fact that diseases of entirely different clinical type may be produced in the two cases.

The epidemiology of certain forms of virus disease remains yet obscure. In herpes simplex the most commonly held view is that the virus, while normally present, becomes active and produces its characteristic lesions only when the natural resistance of the body is reduced by some intercurrent infection (e.g. pneumonia, common cold, &c.). A similar view has been held with regard to the occurrence of encephalitic sequelæ of measles, chicken-pox and vaccination against smallpox, viz. that some latent virus is stimulated to activity by the presence of a concomitant virus infection. This question, however, is still a much disputed one.

Of other factors entering into the epidemiology of virus diseases, season is, in some, of undoubted importance. Poliomyelitis, for example, has its rise and fall in the late summer and autumn, whether in the northern or southern hemisphere, while encephalitis lethargica, whose ætiology is still dubious, prevails in the spring months. In many virus diseases of man and animals, age plays an important role in respect both of incidence and severity. Similar features are noted in diseases of insects such as sacbrood and polyhedral disease, to which the adult insect is usually immune and the larval form only susceptible.

Where plant viruses are concerned, Kunkel (1928) informs us that the final clinical feature (produced by inoculation) is the same whether the infective agent is introduced into old or young tissues. The spread and severity of insect-borne virus diseases, whether of animals or plants, depend also to a very large extent on the natural history and seasonal prevalence of the vectors concerned. The healthy virus-carrier has not, so far, attracted serious attention as a factor of epidemiological importance in the virus diseases of animals, though some interesting data on this question, referred to later, have been placed on record. In plant mosaics, on the other hand, the problem of the healthy virus carrier is of very considerable importance and threatens to interfere seriously with experimental work. Salaman (1929), for example, says, with reference to the variety of potato known as *Kerr's Pink,* that 'it is often difficult to find one plant in a field which has any visible disease in it; when, however, we take cuttings from such plants and graft them on to healthy test plants, they induce mosaic in them'. Other varieties of potato may carry the disease known as Streak without showing any sign of it.

Immunological Problems in Virus Disease.

What degree of immunity to subsequent attack may follow recovery from a virus disease? Statements have been freely made in text-books and discussions on viruses that recovery from a virus attack confers a fairly solid immunity against subsequent infection and that in this matter virus diseases differ from frankly bacterial infections. Sometimes the statement is made only to be promptly qualified by exceptions to the rule. The fact is, however, that no dogmatic comparative statement of this kind is warranted in view of the wide variations in this respect met with both in bacterial and in virus infections. In some bacterial infections, e.g. respiratory or dysenteric disease, immunity to subsequent attack is notoriously feeble, while in others, e.g. enteric fever, second attacks are at least rare. The question of exposure and of increasing natural resistance with age enter largely into this problem. That a person who has had measles once in infancy is not likely to have it again, is not so surprising as the fact that susceptibility to infection diminishes with age. There is the further difficulty presented by the records of second attacks of conveying complete assurance that the second attack was bacteriologically of a similar nature to that of the primary. Among the virus diseases again some, like foot-and-mouth disease, confer only slight immunity of short duration, while others, like small-pox, are believed to confer solid immunity, though the rule is not absolute. It is doubtful even if a virus disease like yellow fever, which was long believed to confer absolute immunity, can still be regarded as an outstanding example of this phenomenon. As in certain bacterial diseases in which the carrier state has been definitely established (e.g. enteric fevers, bacillary dysentery and undulant fever), so also in certain virus diseases (e.g. fowl-pox, plant mosaics, &c.) liability to second attacks, or, alternatively, complete immunity to subsequent attack, would seem to be correlated with the continued presence of the microbe or virus in the tissues. The presence of the virus or bacterium for long periods after the initial infection might equally well determine a hypersensibility or a solid immunity according as the natural defences of the body vary in intensity at different times. In certain virus diseases evidence has been accumulating that the virus may persist in the body for a considerable time after all clinical manifestations have disappeared, e.g. in fowl-pox, experimental vaccinia of rabbits, and possibly experimental poliomyelitis of monkeys, when recovery takes place, as it may rarely do. Recently Olitsky and Long (1929) and Olitsky, Rhoads and Long (1929) have published evidence showing that from the organs of vaccinated rabbits and the cords of monkeys recovered from poliomyelitis, the viruses, though not demonstrable by ordinary methods of subinoculation, may be revealed by cataphoresis of the tissue. These experiments are of great interest, and it is to be hoped that the method will be fully exploited and its limitations ascertained. It is known, for example, that the salivary gland virus of Cole and Kuttner can readily be recovered from immune guinea-pigs.

Do viruses produce antibodies of the ordinary kind in susceptible animals? This question has aroused considerable diversity of opinion and there has been exhibited by writers a very definite tendency to assume that the phenomenon of antibody-production and the behaviour of antibodies *in vitro* and *in vivo* are so thoroughly well known that divergences exhibited by viruses in these respects are particularly worthy of note as differentiating features. In virus diseases antibody-production has been so far only inadequately explored, and in evaluating the data available consideration must be given to the fact that the virus cannot be presented to the antibody either in a pure state or in cultural form as bacteria can be presented. Results indicating positive complement fixation with viruses such as vaccinia, herpes, rabies, foot-and-mouth disease, in their native milieux have been unequivocally obtained by some workers, while others, who have failed (e.g. Schultz and collaborators, 1928), have attributed the recorded positive results to non-specific reactions. Of these positive results one may quote those of Gordon (1925) and Bedson and Bland (1929) with vaccinia, and of Ciuca (1928–9) with foot-and-mouth antigen. The latter was able even to distinguish the different types of foot-and-mouth virus by complement-fixation methods. That properly prepared suspensions of vaccine virus show flocculation in the presence of antivaccinial serum has been claimed by many workers and recently by Gordon (1925) and Burgess, Craigie and Tulloch (1929). Until, however, we are in a position to present purer suspensions of virus or artificially cultivated virus to the action of immune serum, there is not likely to be much unanimity with regard to the precise mechanism of the observed effects.

The antiviral body has, on the other hand, been far more thoroughly studied. In explanation of the neutralization effects noted *in vivo* it was for long believed that the virus suffered some destruction by preliminary digestion *in vitro* with the immune serum, but it would appear that so far as vaccinia is concerned only slight evidence of union of vaccinia virus with antiviral body *in vitro*, and that of a loose character, can be demonstrated (Andrewes, 1928; Long and Olitsky, 1930). Bedson (1928, 1929), on the other hand, has presented good evidence that some form of union of herpes virus with its antiviral body occurs *in vitro*. It may, indeed, be found that the neutralizing effect of antiviral body *in vivo* may be influenced by conditions of preliminary contact *in vitro* and to different degrees with different types of virus.

How far the virus-neutralizing effect of antiviral serum observed *in vivo* is to be attributed to phagocytosis of opsonized virus, which is thus prevented from attacking susceptible cells, or how far an antitoxic action of serum may be involved, is not known. One of us (Ledingham) has followed the behaviour of the Paschen bodies in day-to-day preparations from vaccinial lesions in normal and immunized rabbits and has confirmed the statements of Paschen that in the very early stages of the vaccinial lesion these bodies lie free and in enormous numbers, while in the later stages they congregate in clumps in the neighbourhood of cells of the exudate. At present the exact status of the Paschen body is controversial, and there is so far only a suggestion that the phenomena observed *in vivo* may not be dissimilar from those peculiar to a coccal phlegmon.

The fact that in-vitro union of virus antigen and antiviral body is usually weak has been held to differentiate the antiviral body from other bodies such as bacteriolysins and bactericidins. On the other hand, it is notorious that mixtures of bacteria and their corresponding antisera in which there is little evidence of in-vitro bacteriolysis may behave as neutral mixtures in the living body, where opsonic and phagocytic processes come into play.

Todd (1928) has shown that a neutral mixture of fowl-pest virus and immune serum may be lethal if the volume of fluid in which it is exhibited is greatly increased, and similar phenomena have been recorded by Long and Olitsky (1930) working with vaccinia. These results may, as has been suggested, be genuine dissociation effects in unstable neutral mixtures. When, however, as appears to be the case in vaccinia, the union *in vitro* is of a weak order, it is scarcely appropriate to speak of dissociation. It is just possible that as the total volume of fluid in which the neutral mixture is presented to the tissues is increased, the wider opening-up of lymph channels may favour the dispersion of antibody into the general circulation at the expense of virus. The latter, as we know, effects a union with the susceptible tissues extremely rapidly.

It is probable, therefore, that no very reliable inference as to the different comportment of viruses can be drawn from experience gained in the demonstration of antigen-antibody reactions by in-vitro methods. Some viruses like vaccinia would appear to attack very promptly the susceptible cells, and even slight postponement in the introduction of the antiviral serum may result in failure to arrest the normal action of the virus. This phenomenon, however, has many parallels both with antibacterial and with antitoxic action.

What evidence is there that active immunity can be conferred by dead viruses? It has been held by some workers that killed viruses do not confer active immunity and that where positive evidence of such has been presented, the viruses in the immunizing suspension have not been really dead. They would place, therefore, these viruses in a different category from bacteria, inasmuch as in the dead state they do not act as prophylactic antigens. The assumption, however, that solid immunity against bacterial infections by means of killed vaccines can be postulated as an invariable occurrence is unwarranted (see Vol. VI, Chapter III). If we assume that the viruses as used for prophylaxis in rabies, foot-and-mouth disease, dog distemper, fowl-pest, yellow fever and herpes, are really dead, as seems legitimate from the evidence, the degree and duration of the immunity realized would be quite comparable with that yielded experimentally by killed bacteria such as *B. typhosus*, *B. dysenteriæ*, *V. choleræ* and *B. pestis*. The virus must always be at some disadvantage in such comparative tests owing to the difficulty of securing an adequate amount of it for prophylactic purposes. There is the further consideration that in virus diseases of veterinary importance the use of the attenuated living virus for prophylaxis is practical and often very efficient. Consequently, the possibilities of killed virus have not been sufficiently explored. It remains, however, true that both in bacterial and in virus diseases the best successes in prophylaxis, in so far as degree and duration of immunity are concerned, have been obtained with the living attenuated virus. That the injection of small amounts of killed virus can induce fresh development of antiviral bodies in an animal whose immunity has fallen to low levels is now admitted, so that in this particular feature dead bacteria and dead viruses behave similarly.

One final question concerns the statement that antiviral bodies are produced only in animals naturally susceptible to the virus. How far this statement can be substantiated is doubtful. In some virus diseases, e.g. fowl-pox, it has been difficult to demonstrate the presence of antiviral bodies in the serum of recovered and even of hyperimmunized fowls, but more recent evidence would suggest that these antibodies are really produced, though in small quantity. On the other hand, it has been clearly shown (Fairbrother, 1930) that antiviral bodies can be produced by immunization of the horse with poliomyelitis virus though this animal is naturally insusceptible to the virus.

Cell Changes produced by Viruses and the so-called Cell Inclusions.

A full discussion of the cell changes produced by viruses in the course of their interaction with the host tissues would, to be adequate, involve a description of the various inflammatory, granulomatous, necrotic or proliferative processes characteristic of the various virus infections. These phenomena are dealt with in the special chapters to follow. Here we may simply note that attempts have been made to divide the types of histological change met with in various virus infections into two kinds, one associated with degenerative changes of the cells involved and the other with proliferative processes. Thus, foot-and-mouth disease and variola might be cited as illustrative of the former processes, and the filterable fowl tumours and fowl-pox, of the latter. The division seems, however, to be a purely arbitrary one. In vaccinia and smallpox early degenerative changes in the reticulo-endothelial tissue are usually followed by active proliferation of the same tissue, and at any time the result of the interaction between virus and host cell will be determined by the balance of two forces, dose of virus and natural resistance.

There has been controversy as to the exact point of attack of virus after introduction into a skin site or into the circulating blood. The subject has been studied only in a few outstanding cases. Some have held, for example, that vaccinia virus possesses a definite affinity for tissues of epiblastic origin. It is now, however, generally agreed that this virus proliferates readily in tissues of mesoblastic origin and that the reticulo-endothelial tissue, wherever situated, is the seat of primary attack and takes the chief share in repair. The fact that the epidermal cells of the Malpighian layer are as a rule gravely affected in a vaccinial lesion can readily be attributed to interference with their nutrient supply by vascular inflammatory changes in the dermis below. Even in the case of fowl-pox, where to naked-eye and microscopic appearance, there would seem to be extensive proliferation of Malpighian cells, one of us (Ledingham, 1926) has pointed out that the tumour-like growth is due mainly to enormous swelling of the cells and distortion of their layers by exudate passing outwards from the inflammatory area below (see also Andervont, 1926).

The hyperplastic epithelial plaques which are well-known features of the attack of vaccine virus on cornea have been interpreted as denoting an essential epiblastic affinity though it is admitted that in the sub-epithelial tissue of the cornea inflammatory processes are always in evidence. Teleologically, the formation of the plaque might be looked upon as nature's method of protecting the cornea from ulceration. In any case, we now know from experience with the visceral lesions that may be produced in the rabbit by neurovaccine or neurotesticular vaccine that precisely similar plaques, consisting of proliferated serous endothelium, appear on such organs as the lungs, ovaries, uterine horns and intestine, over the sites of subserosal necrotic lesions (Ledingham and Barratt, 1929). Their occurrence in these situations would, therefore, lend no support to the view that cells of epiblastic origin are specially prone to infection.

With regard to foot-and-mouth disease and poliomyelitis, histological evidence (Galloway and Nicolau, 1928) would suggest that in the former the epithelia are primarily attacked and in the latter the anterior horn cells (Hurst, 1929).

Cell inclusions. A separate chapter is devoted to these structures, where views as to their nature and origin are discussed. Here it may be said that so far it has not been possible to produce similar structures by other means than the particular virus concerned. To that extent they are of specific importance. While the morphology of the inclusion is quite specific in so far as the Guarnieri body, the Bollinger body and the Negri body are concerned, there are other types of inclusion, notably the intranuclear acidophile body, which is only virus-specific and not type-specific. Such bodies are found in a number of virus diseases, and not alone in cells of epithelial origin (e.g. herpes, chicken-pox, yellow fever, virus III). There is considerable support for the view that the specific cell inclusions afford a valuable clue to the presence of an associated virus and the fact is also appreciated in the study of plant viruses. Recent work (Andrewes, 1929 ; Rivers, Haagen and Muckenfuss, 1929[1]) shows that the development of characteristic cell inclusions may also be usefully employed as an index of growth of virus *in vitro*.

There remains for consideration the nature of the minute granules (elementary corpuscles), such as the Paschen bodies, demonstrable in vaccine lymph and those small particles into which the Bollinger body in fowl-pox can be resolved by appropriate methods of fixation and staining.

Woodruff and Goodpasture (1929) have shown that by the action of trypsin on the fowl-pox lesion, it is possible to isolate the Bollinger bodies from their surroundings and even to infect fowls with a single body. In view of the filterability of the fowl-pox virus it seems highly probable that the infective agent in such a case was the intracorpuscular particle. In spite of the minute size (about $0 \cdot 2\mu$) of the Paschen and other elementary bodies, it may yet be possible to submit them to accurate study with a view to establishing their possible causative relationship to the virus disease.

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CHAPTER II. CELL INCLUSIONS.

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The Problem of Cell Inclusions.

Within certain cells of organisms infected with some filterable virus diseases there occur atypical bodies, or granulations, which are known as ' cell inclusions ', or ' virus bodies '. In some diseases, such as rabies and fowl-pox, they occur with such constancy, and are so characteristic as to be of diagnostic value. In other virus diseases investigators have described cell granules of doubtful nature as specific virus inclusions, without proof that they are the result of such infections.

The interpretation of virus bodies is a matter of considerable difficulty owing to the incompleteness of our knowledge of the exact character of viruses. It is not definitely established that filterable viruses are living organisms, and although they seem incapable of multiplying except in the presence of living cells, we do not know whether they penetrate cells, or exert their influence owing to the penetration through the cell membrane of products of their metabolism. The result of their activity is seen in the proliferative changes of fowl-pox and warts ; but cell degeneration may be their predominating effect, as in varicella.

Theories as to the Nature of Cell Inclusions.

The various theories that have been put forward to explain virus bodies fall into three groups :

(1) That they represent organisms, or special stages in the life-cycle of organisms. When virus inclusions were first discovered they were regarded by many observers as protozoa. The Guarnieri bodies of variola and vaccinia were given the name of *Cytoryctes vaccinæ*, and an elaborate life-cycle was attributed to this supposed organism. Those who have upheld the protozoan theory of virus inclusions have often been misled by their staining reactions. With a stain such as Mann's methyl-blue-eosin, the cytoplasm and true nucleoli (plasmosomes) of normal cells stain red with eosin, the chromatin and chromatin nucleoli (karyosomes) blue with methyl blue. Certain virus bodies such as those of vaccinia and rabies show a double staining of the same kind. The inclusion as a whole stains red, but contains blue-stained granules. Since similar results can be obtained with other dyes, some investigators have assumed that the inclusion bodies contain chromatin, the same as typical protozoa. Recent researches have shown, however, that ordinary staining reactions cannot be relied upon as a test for chromatin (Ludford, 1928[1]), and up to the present time no chromatin has been demonstrated microchemically in any cell inclusion.

(2) That they represent organisms together with the product of cellular reaction towards infection. When it was found that the infective agents of virus diseases were small enough to pass through porcelain filters, it became difficult to reconcile this fact with the protozoan theory. Von Prowazek (1907) has suggested that filterable viruses are organisms which have two stages in their life-history, an extracellular, and an intracellular one. ' Elementary bodies ' he calls the extracellular stage. These are sufficiently small to pass through porcelain filters. When they enter cells they become ' initial bodies '. They proliferate and become coated with products of reaction of the infected cells. On disruption of the host-cells they are set free, and as ' elementary bodies ' are capable of spreading the infection. The term ' Chlamydozoa ', meaning ' cloak animals ', was applied to these hypothetical organisms. Lipschütz calls these minute granular organisms of von Prowazek ' Strongyloplasmata ' (pebble-plasms).

(3) That they represent products of cellular reaction to infection, or are the result of cell degeneration. In only one case has it been definitely proved that virus bodies are incapable of spreading the infection. Glasser (1928) was able to show that the intranuclear polyhedra of virus diseases of insects alone are unable to induce the disease (Fig. 5, w).

There is so much difference between the various kinds of virus bodies that it is highly improbable that any one theory is capable of embracing them all.

Distribution.

Virus bodies have been described in cells of plants, insects, fish, amphibians, birds and mammals. In most virus diseases the inclusions show a marked tendency to be restricted to one particular type of cell. The Negri bodies of rabies occur only in nerve-cells, although they may be seen in sections lying outside neurones. The peculiar inclusions in lymphocystic disease of fish are confined to connective tissue-cells. The intranuclear bodies of guinea-pigs are restricted to the salivary duct cells. The Bollinger bodies of fowl-pox are seen only in epidermal cells. The intranuclear polyhedra of virus diseases of insects are not limited to only one tissue. According to Glasser (1928) polyhedra are seen in nuclei of hypodermal, fat, and tracheal matrix-cells, and also in certain blood-cells. In infectious myxomatosis of rabbits inclusions have been described in epidermal, and also in connective tissue-cells.

It is significant that by far the largest number of inclusions occur in the epidermis, a tissue which is avascular, and the cells of which, apart from the basal layers, are disintegrating in the course of keratinization. Avascularity alone implies that cells have not the usual means of ridding themselves of waste products. At the onset of keratinization the cellular mechanism of segregation and elimination breaks down, thus products of metabolic activity accumulate. Then the nucleus degenerates. Nucleolar material is extruded into the cytoplasm, where it swells up and contributes towards the keratohyalin granulation of the *stratum granulosum*. Finally the protoplasm becomes converted into the scleroproteid, keratin (Ludford, 1924–5). During this disintegration all kinds of granules can be stained in the cells, offering a wide field of choice to seekers after virus bodies who are not familiar with normal cytological processes.

It is obvious that if a virus stimulates the formation of certain products of metabolism, or results in new substances being formed, they are more likely to accumulate in epidermal cells than in others. Such may be the explanation of the origin of the ' acidophil ' nuclear inclusions of many virus diseases, as those of the herpes group, and varicella. There is the further possibility that the disintegrating cells of the epidermis constitute a favourable medium for the growth and development of living filterable viruses.

Classification.

In the absence of definite knowledge of the nature of virus bodies, a satisfactory scientific classification is impossible. Lipschütz (1921) separates them into three groups according to where they occur in the cell :

(i) Inclusions in the cytoplasm.
(ii) Inclusions in the nucleus.
(iii) Inclusions in both the cytoplasm and the nucleus.

This classification has obvious disadvantages, since, as Jackson Clark (1928) points out, it involves separating two such closely allied diseases as vaccinia and variola. Nevertheless, in the absence of a more satisfactory method, it has been adopted by subsequent writers. Rivers (1928) has used it in drawing up a list of diseases in which intracellular inclusions have been described. In the following table his arrangement has been modified so as to separate diseases ' in which intracellular changes are sufficiently characteristic to be significant ' from others which have been described at various times, and need further confirmation. The latter follow the word *also* in each division.

Diseases in which Cell Inclusions have been described.

(i) *Inclusions in Cytoplasm.*

Vaccinia (Cow-pox).
Sheep-pox.
Fowl-pox (contagious epithelioma).
Molluscum contagiosum.
Rabies.
Lymphocystic disease of fish.
Infectious myxomatosis of rabbits.
Mosaic diseases of plants.

Also—Distemper of dogs, fowl plague, lethargic encephalitis, trachoma, hog cholera, South African horse sickness, measles, scarlet fever, diseases of fowls in Nigeria and Palestine, contagious pustular stomatitis of sheep, Grahamella (moles). (Kurloff bodies of guinea-pigs and Todd bodies of frogs.)

(ii) *Inclusions in Nucleus.*

Acidophil or Oxyphil Inclusions.	Herpes group of diseases. Varicella. Virus III infection of rabbits. *Salivary gland disease of guinea-pigs.* Borna disease of horses (Enzootic Encephalomyelitis). 'Visceral disease' (see von Glahn and Pappenheimer, 1925). [Yellow fever (see Torres, 1928)].

Polyhedral disease of caterpillars.

Also—epithelioma of fish, carp-pox, frog-wart (*Discoglossus*), warts, condyloma acuminatum, psoriasis.

(iii) *Inclusions in both Cytoplasm and Nucleus.*

Variola (see Councilman, Magrath and Brinckerhoff, 1904).
Paravaccinia (see Lipschütz, 1919–20).

The Principal Types of Cell Inclusions.

Within the limited space of this article it will be impossible to describe the large number of inclusions which have been recorded up to date. For the sake of brevity the more significant of them will be grouped together for the purpose of describing some of their cytological features. Fuller particulars will be found in the pictographic review by Findlay and the present writer (1926), and in the reviews of Rivers (1928) and Cowdry (1928).

Vaccinia-variola type of inclusions.

(*a*) Without accompanying nuclear inclusions :
- Vaccinia.
- Sheep-pox (Fig. 1, h).

(*b*) With accompanying nuclear inclusions :
- Variola (Councilman, Magrath and Brinckerhoff, 1904).
- Paravaccinia (Lipschütz, 1919–20).

The characteristic cytoplasmic inclusion of these diseases is of the Guarnieri-body type. Renaut (1881) first described spherical globules within epidermal cells of variolous lesions, but Guarnieri (1892) was the first to figure the intracellular bodies which bear his name. The various views that have been expressed as to the nature of these inclusions may be summarized as follows :

(*a*) That they represent a protozoan parasite—a view unacceptable to most modern workers.

(*b*) That they represent phagocytosed red, or colourless, blood-cells, Woodcock (1921)—unsupported by recent work.

(*c*) That they are accumulations of the actual virus, Böing (1920).

(*d*) That they are caused by extruded nuclear material, Ewing (1904–5) —or nucleolar extrusions, Ledingham (1925).

(*e*) That they are due to stimulation of the cell by the virus, resulting in the excessive formation of a substance normally present in the

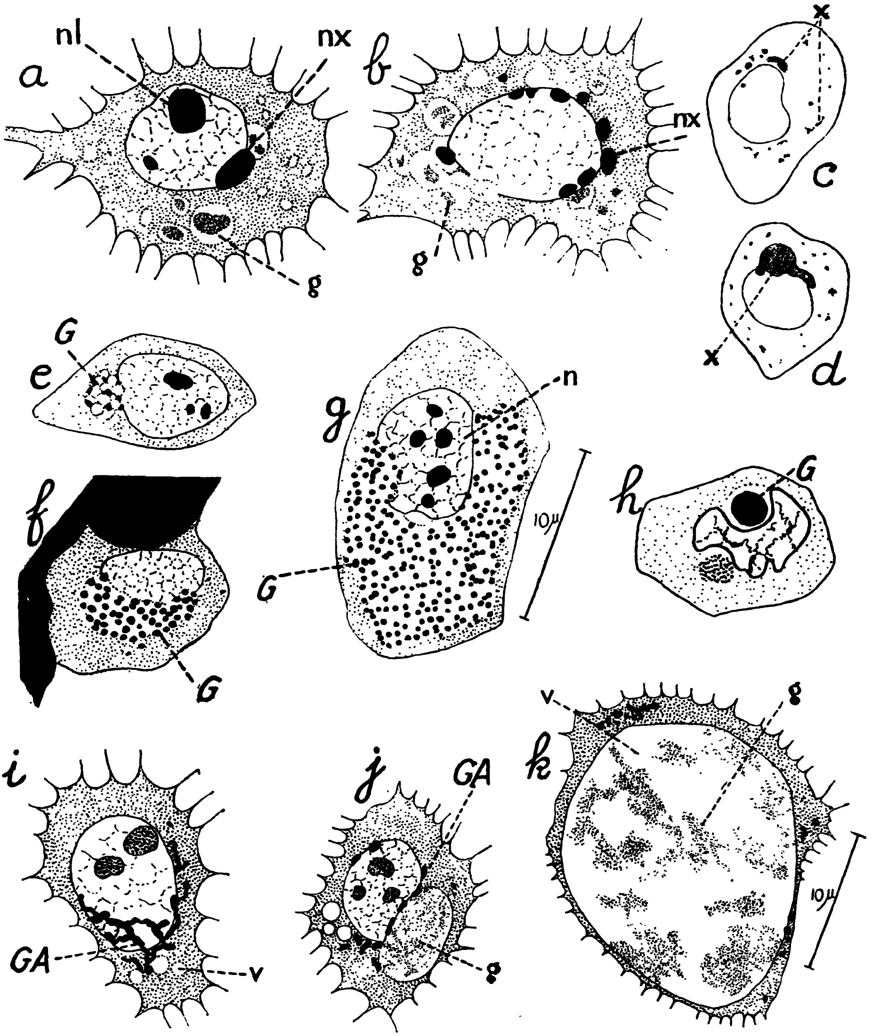

FIG. 1. *Cell Inclusions in Vaccinia and Sheep-Pox.*

a, b. Epidermal cells of cornea of the rat scarified with vaccinia virus, showing nucleolar extrusion (nl, nucleolus : nx, nucleolar extrusions : g, granular material in cytoplasm), after Ludford (1928[2]).

c, d. Formation of Guarnieri bodies from material (x) normally present in the cell but increased in amount by the action of the vaccinia virus, after Cowdry (1922).

e to g. Development of granular structures (G), Guarnieri bodies of Schütz, within epidermal cells immediately surrounding the centre of a vaccinia lesion in the cornea of the rat, after Ludford (1928[2]) (n, nucleus).

h. Guarnieri bodies (nucleolar extrusions) in corneal epidermal cell of sheep with sheep-pox, after Ludford (1928[2]).

i to k. Formation of a finely granular material (g) within vacuoles of epidermal cells of the rat cornea scarified with vaccinia virus (GA, Golgi Apparatus: V, cytoplasmic vacuoles), after Ludford (1928[2]).

cytoplasm, Cowdry (1922), Fig. 1, c and d. According to Cowdry (1928) they exhibit the following reactions:

(i) Stain supravitally with neutral red, janus green, and cresyl blue. (ii) Do not give a positive Millon's reaction, oxydase reaction, or Bensley-Macallum test for masked iron. (iii) Are not blackened by osmic acid, or stained by Sudan III. (iv) Are readily dissolved by 1·5 per cent. acetic acid.

Schütz (1925) has described granular inclusions which he terms Guarnieri bodies in epidermal cells of vaccinia lesions in the rabbit. These inclusions appear to be different from the Guarnieri bodies of other writers. They occur only in epidermal cells immediately surrounding the centre of the lesions. Their mode of development is shown in Fig. 1, e, f, g. Ludford (1928[2]) found that in addition to these granules the typical Guarnieri bodies of other authors are widely distributed throughout the epidermal cells of the vaccinia lesion. They are strikingly similar to nucleolar extrusions, as is seen in Fig. 1, a and b. Further, within epidermal cells of the rat cornea a granular material has been described which is deposited within vacuoles formed in close relationship with the Golgi apparatus, Fig. 1, i, j, k. The size of the particles of this material is much nearer the order of magnitude of an actual virus organism than any of the other inclusions so far described in these diseases.

The molluscum type of inclusion.

Fowl-pox (contagious epithelioma).
Molluscum contagiosum.

Although these diseases are quite different, the inclusions are remarkably alike. Of all the virus inclusions that have been described these most strikingly resemble accumulations of actual organisms.

The inclusions of fowl-pox are named after Bollinger (1873), who seems to have been the first to have studied them histologically. Michaelis (1903) showed that they stained with Scharlach R, and osmic acid. Borrel (1904), in smear preparations of epidermal cells, succeeded in staining minute coccoid bodies. They are accepted by Lipschütz (1921–2) as the actual organisms of fowl-pox. Ludford and Findlay (1928) found that the earliest indication of infection of an epidermal cell is the formation of a small vacuole, to the periphery of which small granules are attached (Fig. 2, a). Mitosis is of frequent occurrence in such cells (Fig. 2, b). These virus vacuoles increase in numbers and become enlarged, at the same time they become surrounded by a lipoidal shell, and present a granular appearance internally (Fig. 2, c). The infected cells enlarge, and the virus bodies coalesce to form a single large granular inclusion (Fig. 2, d).

While this article was in the press a paper was published by Woodruff and Goodpasture (1929) in which is described a method of isolating single virus bodies of fowl-pox, after tryptic digestion of infected epidermal cells, by means of the micro-injection pipette technique of Chambers. 'A single inclusion body, when washed with saline and inoculated into the skin of the hen, has produced a typical fowl-pox lesion containing the characteristic inclusions'. The saline in which the inclusion body was finally suspended before injection was innocuous.

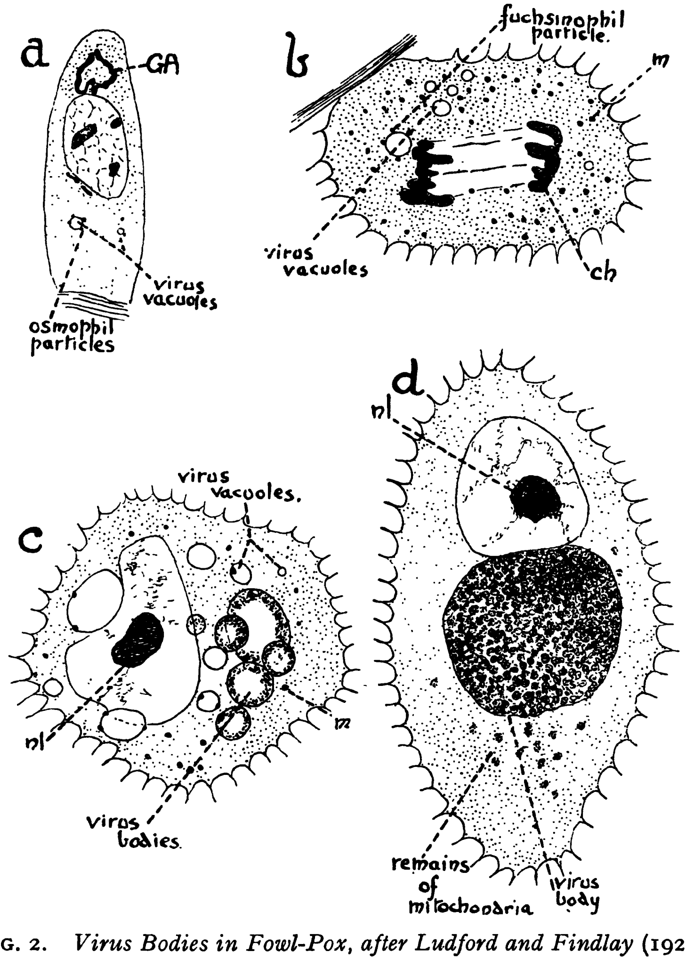

FIG. 2. *Virus Bodies in Fowl-Pox, after Ludford and Findlay* (1926).

a. Epidermal cell at an early stage of infection (GA, Golgi apparatus).

b. Telophase of mitosis of a cell at an early stage of infection, with minute granules at periphery of virus vacuoles stained with acid fuchsine (ch, chromosomes: m, mitochondria).

c. Epidermal cell with numerous inclusions, the larger containing peripheral granulations (m, mitochondria: nl, nucleolus).

d. Epidermal cell with a single virus body composed of granules (nl, nucleolus).

The virus inclusions of molluscum contagiosum according to the recent account of Goodpasture and King (1927) are almost identical with those of fowl-pox, except that they are not surrounded by a lipoidal covering.

The inclusions of rabies.

The view that the Negri bodies of rabies represent micro-organisms has the support of a few workers at the present time. Levaditi, Nicolau and Schoen (1924) believe these inclusions to be composed of accumulations of granules which represent the micro-organisms of the rabies virus, and Manouélian and Viala (1924) consider the parasites to be rod-shaped bodies which agglutinate to form typical Negri bodies. Morphologically two types of inclusions have been described in rabies :

(*a*) The ' lyssa bodies ', which Goodpasture (1925) believes are minute hyaline granules produced from the neurofibrils by the action of the virus (Fig. 3, b and c).

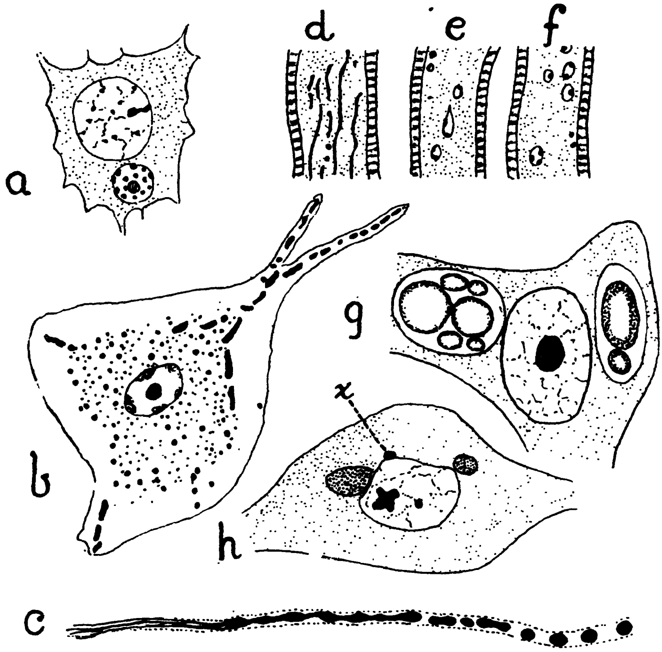

FIG. 3. *The Inclusion Bodies in Rabies.*

a. Negri body in nerve-cell of Ammon's horn of the dog, after Hartl (1927).

b. Ganglion cell showing neurofibrillar material giving rise to bodies identical with ' lyssa bodies ', after Goodpasture (1925).

c. An axis cylinder showing a complete series of changes resulting in the formation of hyaline bodies from neurofibrils, after Goodpasture (1925).

d, e, f. Mitochondria and Negri-body formation, after Goodpasture (1925) : d, shows the mitochondrial filaments ; e, the transformation of mitochondria into globules ; and f, granules on the globules stainable with methylene blue.

g. Two large vacuolated Negri bodies with primary and secondary vesicles in a ganglion cell of the hippocampus major of the bullock, after Acton and Harvey (1911).

h. Ganglion cell of the hippocampus major of the guinea-pig, showing a nucleolar extrusion (x) and two Negri bodies, after Acton and Harvey (1911).

(*b*) The typical Negri bodies (Fig. 3, a). These are usually described as consisting of a ground-substance containing a central body, surrounded by chromatoid granules. The evidence against the view that they represent micro-organisms may be summarized briefly as follows :

(i) They are extremely rare, usually absent, in animals infected by a

' fixed virus '. (ii) They ' vary in shape, size, and staining reactions in the different animals examined, whilst remaining constant for the same animal '—Acton and Harvey (1911). These investigators regard them as products of interaction between nucleolar extrusions and the cytoplasm (Fig. 3, g and h). Besides the rabies virus they consider other agents may induce their formation. (iii) According to Goodpasture (1925) they arise ' by a focal degeneration of neurofibrillar material which melts together and coagulates or coheres ' about one or more structures which result from mitochondrial degeneration (Fig. 3, d, e and f).

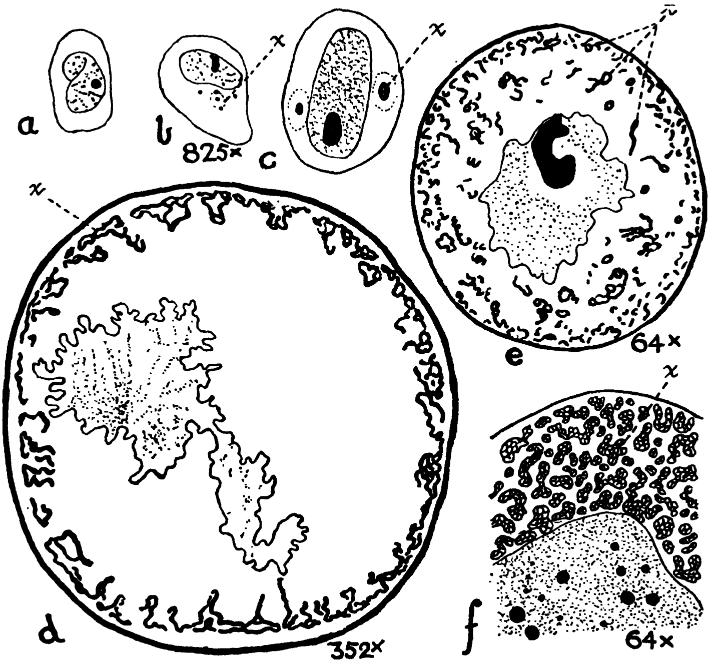

FIG. 4. *Inclusion Bodies in Lymphocystic Disease of Fish, after Weissenberg* (1920).

a. Infected connective-tissue cell with cytoplasm rounded-off (Stone perch).
b. Infected cell containing deeply staining cytoplasmic granules (x).
c. Cell with two virus bodies (x).
d. Section of a medium-size lymphocystic cell 175μ in diameter: virus inclusions (x) reticulate in form.
e. Section of a mature lymphocystic cell of 660μ diameter with reticulate inclusions (x) spread throughout the cytoplasm.
f. Part of a section through a mature lymphocystic cell of 1·4 mm. diameter, showing the virus bodies (x) (Flounder).
All figures more diagrammatic than the originals.

The reticulate inclusions of lymphocystic disease of fish.

The cell inclusions in this disease are unique because of their curious reticulate form and their relatively enormous size. According to Weissenberg (1920) a connective tissue cell which is infected becomes rounded off (Fig. 4, a). Within its cytoplasm basophil granules appear, which enlarge (c), and finally become reticulate. They spread out in the

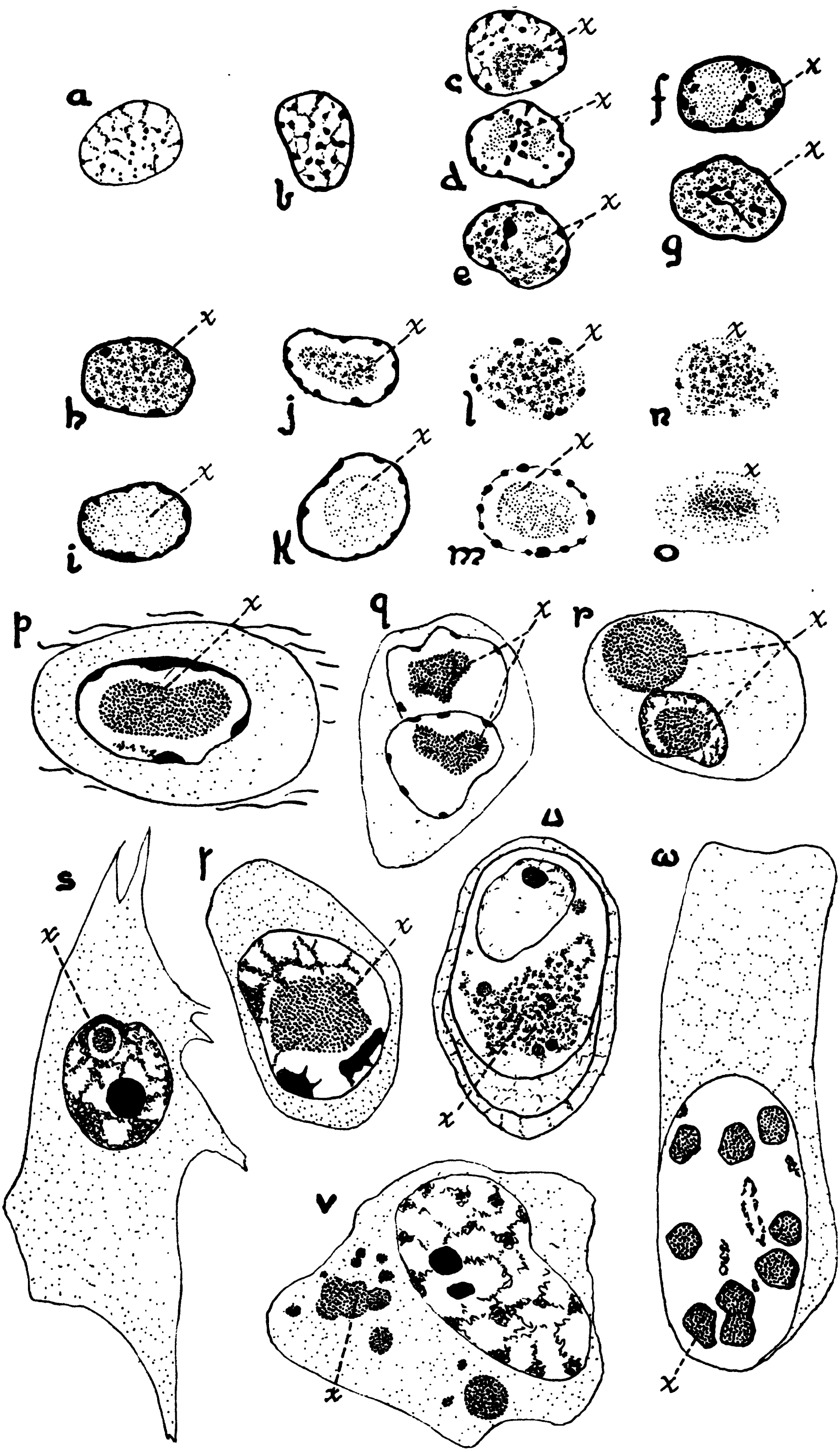
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FIG. 5. *Intranuclear and Cytoplasmic Virus Bodies.*

a to g. Oxychromatic nuclear degeneration, after Lauda and Luger (1926). a to g, initial stages: h to k, fully-developed stages; l to o, end stages. The oxyphil, or acidophil nuclear material, is marked 'x'. (Schematized from the coloured originals.)

p. Intranuclear inclusion (x) in epidermal cell in herpes zoster, after Lipschütz (1921).

q. Intranuclear inclusions (x) in a binucleate endothelial leucocyte from the testis of a rabbit inoculated with Virus III, after Rivers and Tillett (1924).

r. Intranuclear and cytoplasmic inclusions (x) in paravaccinia, after Lipschütz (1919–20).

s. Neuron of the anterior horn of the spinal cord of the lumbar region of a rabbit inoculated with the virus of enzootic encephalomyelitis (Borna disease). Intranuclear inclusion (x), after Nicolau and Galloway (1928).

t. Swollen epithelial cell containing intranuclear inclusion (x) from the submaxillary gland of a guinea-pig, with 'salivary gland' disease, after Cole and Kuttner (1926).

u. Epithelial cell of the rabbit affected by the virus of infectious myxomatosis. The cytoplasm contains a granular mass in the midst of which are several coccoid bodies, after Rivers (1928).

v. Macrophage from the dermis of a rabbit with infectious myxomatosis. Granular inclusions (x) in the cytoplasm. Original, from a preparation by Dr. G. M. Findlay.

w. Intranuclear polyhedra (x) in fat-cells of silkworm, infected with 'grasserie', after Paillot (1926).

cell (d), ultimately extending throughout the cytoplasm (e) and (f). During their development the cell undergoes tremendous enlargement, and its membrane thickens. The infected cell ultimately disintegrates, and the overlying epidermal cells proliferate and grow into the cavity thus formed.

The inclusions of infectious myxomatosis of rabbits.

Both the epidermis and dermis are involved in this disease, and inclusions have been described in both tissues. In the cytoplasm of epidermal cells, Rivers (1928) has figured granular eosinophil material surrounding basophil granules (Fig. 5, u). In the connective tissue-cells Lipschütz (1927) found minute irregular coccoid-like bodies which he regards as a new type of organism, and calls *Sanarellia cuniculi* (Fig. 5, v).

Acidophil or oxyphil intranuclear inclusions.

Unaccompanied by cytoplasmic virus bodies :
  Herpes group of diseases (Fig. 5, p).
  Varicella.
  Virus III infection of rabbits (Fig. 5, q).
  Salivary-gland disease of guinea-pigs (Fig. 5, t).
  Borna disease of horses (Enzootic Encephalomyelitis) (Fig. 5, s).
  ' Visceral disease ' (von Glahn and Pappenheimer, 1925).
  [Yellow fever (Torres, 1928)].

Accompanied by Guarnieri-body type of inclusions in the cytoplasm :
  Variola (Councilman, Magrath and Brinckerhoff, 1904).
  Paravaccinia (Fig. 5, r, Lipschütz, 1919–20).

The intranuclear virus bodies found in the above diseases are remarkably alike. Although some occasionally stain faintly basophil, the majority stain readily with acid dyes, such as eosin and acid fuchsine. Lipschütz (1921) regards these bodies as being composed of virus organisms embedded in nuclear material. According to Goodpasture (1925) they are indistinguishable from material precipitated in the normal nucleoplasm by Helly's fluid. To Lauda and Luger (1926), however, they are degeneration products resulting from what they call 'oxychromatic' degeneration. This process as they conceive it to happen is represented somewhat diagrammatically in Fig. 5, a to o. A normal nucleus is shown at 'a'. It becomes hyperchromatic (b). Acidophil material is then deposited which appears either granular (c), homogeneous (d), or both (e). The acidophil material increases in amount (f) and (g), and the whole of the chromatin becomes peripherally arranged (h) and (i). The basichromatin gradually disappears (j, k ; l, m) and ultimately acidophil bodies remain, which may be granular (n) or practically homogeneous (o). Cowdry (1928) has recently made a microchemical investigation of some of these intranuclear inclusions. He finds : (i) they do not give the Bensley-Macallum test for masked iron ; (ii) they fail to stain by Feulgen's method for demonstrating chromatin (thymus-nucleic-acid-protein complexes) ;

(iii) they are insoluble in mixtures containing large amounts (80 per cent.) of acetic acid and chloroform, also in 95 per cent. ethyl alcohol; (iv) they do not give a positive Millon's reaction.

Further Outlook.

The preceding paragraphs will have emphasized the unsatisfactory character of our knowledge of inclusion bodies. Not even in one case do we know the exact chemical composition of the substance constituting the inclusion. We may expect increase in our knowledge of this subject by:

(*a*) The fuller application of the present microchemical (histochemical) methods, and by the discovery of new methods for demonstrating products of cell metabolism.

(*b*) Detailed analyses of the action of viruses on the various cell organs, nuclear and cytoplasmic.

(*c*) Investigating further the possibility of producing 'inclusion bodies' with non-living reagents.

(*d*) The employment of microscopical equipment making possible the use of shorter wave-lengths than those of the visual spectrum, thus increasing the power of microscopic resolution.

Advances in experimental cytology have placed in the hands of investigators a wide range of new methods—vital staining, tissue culture, microdissection and injection—which, in this field, have not been exploited to their fullest extent.

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CHAPTER III. VIRUS DISEASES OF PLANTS.

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Introduction.

The first demonstration of the existence of filterable viruses, either in plants or animals, was given in 1892 by Iwanowski, when he showed that the agent causing the mosaic disease of tobacco passes through a Chamberland filter-candle. Since that date our knowledge of what constitutes a virus disease has been greatly extended, and it is now recognized that viruses occur throughout a very wide range of flowering plants, and are responsible for some of their commonest and most important diseases. In relatively few instances has filterability been proved. In most cases infection, though it can be conveyed in other ways, cannot be transmitted by the artificial inoculation of extracted sap or juice, and there can be no direct evidence of the filterability of the virus. The characters of a virus disease, however, are sufficiently distinctive in plants without such evidence. The nature of the symptoms and the site and manner in which they develop in the plant, combined with the method of transmission and the failure to find any other demonstrable cause, are usually enough to decide definitely whether or not a disease is to be included in the virus group, and it is only rarely that there is any real doubt, when once its characters are fully known.

Virus diseases are found in monocotyledonous and dicotyledonous plants in both tropical and temperate climates, in families of the most diverse character and distant relationship, and in species whose number, already very large, is continually being added to*. They are most common in herbaceous, but occur also in woody, plants. They are found in weeds and in many wild plants, but have been most studied in commercial crops, in some of which their effects are very serious. The combined loss due to virus disease alone in tobacco, sugar-cane, potatoes, sugar-beet, cucumbers —to name only a few examples—amounts every year to several million pounds, and in some places the severity of epiphytotic attack has been so great as to threaten the extinction of the local industry. In this respect virus diseases are of greater importance than they were a generation ago. The increase is to be attributed to changes in agricultural and horticultural practice rather than to a development of viruses not previously existing. The search for new and more profitable varieties, aided by greater knowledge, greater intercommunication and greater facilities of transport, has introduced into fresh localities diseases from which the existing crops were previously free. It may be, of course, that actually new viruses have appeared, but there is no clear proof that this has occurred. It is not often possible to identify with certainty a disease from descriptions in the older literature, but we have reliable evidence that virus diseases were known and common in potatoes in this country at least as early as 1778 (Anderson, 1792), and it is probable that the increase of recent years is due mainly, if not entirely, to the introduction of old diseases into new areas and new hosts.

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* At the end of 1928 virus disease had been reported in 49 families, 167 genera and 264 species of plants.

Symptoms.

Virus diseases in plants are as a rule systemic diseases. Instances are known where an infection remains localized to the point of inoculation, e.g. stem inoculation of *Datura stramonium* with tobacco mosaic (Johnson, 1926[2]), but they are exceptional. In the great majority of cases the disease spreads throughout the entire plant, including root, flowers and fruit, no matter where the infection has entered ; but the distribution is not uniform, there being a greater virus concentration in some parts, e.g. chlorotic areas, than in others. The spread may take place very rapidly, and the presence of virus has repeatedly been demonstrated before symptoms have developed. In curly-top of beets the virus was found to travel from 10 to 21 cm. along the petiole in half an hour (Severin, 1924), and in a streak of maize 40 cm. through the leaf in two hours (Storey, 1926). In both of these cases infection was transmitted by insects and the virus may have been injected directly into the phloem. Leaf-roll from a graft has been shown to travel downwards in the potato 48 cm. in 15 days (Murphy and McKay, 1926[1 & 2]). After inoculation of infective juice into a leaf, tobacco mosaic virus took over three days to reach the stem (Allard, 1914; Böning, 1928), while in cucumber mosaic all parts of the stem and leaf may be infected in the same time (Doolittle, 1920). The high speed in some of these cases suggests that distribution occurs through the vascular system, and it is not unusual to find the first symptoms appearing along the veins ; but the possibility of infecting through the trichomes, e.g. in tobacco mosaic, shows that some viruses at least are capable of passing through other tissues.

The virus, however, does not produce symptoms everywhere throughout the plant. It is the general rule, though to this also there are exceptions, that parts of plants which were already mature at the time of infection do not show visible signs of the disease. The signs normally appear only on parts which are still in course of development, and most commonly and most markedly on the younger parts, which appear after infection has occurred. This is in accord with the general impression that viruses increase most actively in the presence of growing or dividing tissue. It suggests that they exert their effects mainly on growing cells, and that the symptoms themselves are secondary to an interference with the normal development of the tissues. This is supported by the histology of the growing leaf infected in its early stages. In affected portions of the mosaic leaf of tobacco, for example, the differentiation into the palisade cells, which form the greater part of a normal leaf, may have failed more or less completely to take place, and in their stead is a tissue in which the intercellular spaces are small, the cells are isodiametric, and the chloroplasts are abnormal in position, shape and content (Iwanowski, 1903; Dickson, 1922; Goldstein, 1926).

The most frequent symptom is chlorosis, the visible sign of the degradation of the chlorophyll. It may take the form of a general yellowing of the foliage, the so-called 'yellows' group of diseases. More usually it appears as a mottling of the leaves, a blotching of lighter green, or yellow, or even white, irregularly distributed on the darker background of the green leaf. When this is a prominent symptom the disease is known as a mosaic, and the mosaics form the largest group of plant virus-diseases. The blotches or spots may be sharply delimited or blend insensibly into the surrounding tissues, and in certain diseases the pattern of the mottle is fairly characteristic. Sometimes, e.g. in tobacco ring-spot, it takes the form of very striking concentric rings, each pale ring separated from its neighbour by a zone of nearly normal colour. There is usually some stunting of growth and often some degree of malformation. When the stunting is severe, with great shortening of the internodes, the leaves are crowded and bunched together, producing a rosette appearance which may be very characteristic, e.g. the rosettes of pea-nut, wheat and peach. A less frequent symptom is necrosis, which may occur with or without marked chlorosis. This may show itself as spots upon the leaves or as stripes upon the stems and petioles, or may occur in the deeper lying non-chlorophyllous parts of the plant, especially in the phloem.

The virus rarely kills the plant outright. It usually enfeebles it, reducing the growth and lowering the yield, and in some cases the plant may fall a victim to secondary fungal or bacterial parasites; but as a rule the plant survives and sometimes with very little impairment of vitality. Recovery is unusual. Many of the recorded cases are instances of a masking of the symptoms, but in sugar-cane it is not infrequent and other apparently authentic cases are reported. Such plants are not immune to reinfection, and there is no known case of the development of immunity in a plant after an attack of virus disease.

The appearance of the symptoms is markedly affected by external conditions. A potato inoculated with mosaic and kept at a temperature of 24° C. or higher may develop no signs of the disease. Even when the symptoms are already well marked, removal to an air temperature of 24° C. is followed by their gradual disappearance in about a week, and the developing new growth comes out unmottled. Even intermittent exposure to the high temperature produces the same effect but more slowly. The plant is not cured, and removal to a lower temperature brings a reappearance of the symptoms. With tobacco mosaic the temperatures necessary to produce masking are higher, viz. 36 to 37° C. Further, there is a range of temperature which is most favourable to the development of signs : in tobacco 28 to 30° C., in potato 14 to 18° C. (Goss and Peltier, 1925 ; Johnson, 1921 and 1922 ; Tompkins, 1926). To some extent, no doubt, this favourable range is conditioned by the temperature which gives the best growth of the plant. It is a familiar observation that in many virus diseases the symptoms develop only slowly and imperfectly, or may even be suppressed, when a plant is growing slowly, as in winter. But it is not yet clear whether the effect is due to an immediate action of the temperature or other condition on the virus itself or indirectly to its effect upon the host, e.g. its growth-rate.

The symptoms are commonly reported to be less marked in plants grown in shade, but observation under controlled conditions has shown that variations in the intensity of light have very little effect on the mottling of mosaic (Goss and Peltier, 1925 ; Tompkins, 1926). Red light has also only a slight effect, but when either whole plants or individual branches are grown under blue hoods, the signs are very much less marked and may be suppressed in the hooded parts (Lodewijks, 1910 ; Chapman, 1917 ; Dickson, 1922). In this case the masking is more likely to be due to the effect upon the host than upon the virus itself.

While, then, the symptoms may in certain cases be suppressed or caused to disappear by modification of the environment, there are other instances in which no signs of the disease appear at all. The plants are fully infective and readily transmit the disease to susceptible hosts, but they themselves are tolerant, growing normally and giving a normal yield and remaining free from any symptoms throughout their life. A number of such ‘ carrier ’ cases has been recorded. Most of these are doubtful and have been insufficiently investigated, but there are a few instances where the evidence is convincing. For example, the Up-to-Date variety of potato frequently carries streak, a deadly infection to many potatoes, without showing any signs of its presence, but the disease is readily transmitted by grafting to an intolerant variety such as Arran Victory. In hops, also, some varieties are genuine carriers of mosaic (Salmon and Ware, 1928). The danger to commercial production caused by the existence of such susceptible tolerant varieties needs no emphasis. At one time it was suggested that virus disease had been originated by the inoculation into suitable hosts of material derived from normal plants. Johnson (1925) found that the introduction of crushed foliage from seemingly normal potatoes into normal tobacco produced mosaic disease which had all the characteristics of true virus disease. It is most probable, however, that the potatoes used in these experiments were not really normal, and already contained the mosaic, without its presence being detected. When the experiments were repeated with potatoes proved to be free from virus, the phenomenon was not obtained (Henderson Smith, 1928^2). There is up to the present time no satisfactory evidence of the origin *de novo* of any plant virus.

Transmission.

Transmission through seed is on the whole uncommon. It occurs with the mosaic of lettuce and the mosaics of a number of leguminous plants, e.g. the garden pea, three kinds of bean and several clovers. Even in so highly infectious a disease, however, as tobacco mosaic, where all parts of the plant, including the flowers, contain the virus, it is not carried through the seed, and this is the general rule. A satisfactory explanation of this striking fact has not yet been given, but it may be noted in this connection that virus disease usually reduces the number of seeds and sometimes causes sterility.

Under natural conditions infection is commonly transmitted by insects, usually by sucking insects such as aphids, but also by crushing or biting insects. Transmission through soil has been demonstrated with certainty in only one case, the rosette of winter wheat (Webb, 1927), and, even in this, insect intervention has not been excluded. Transmission by simple contact is not known, and in all cases a wound of some kind is necessary. Artificially, transmission may be effected in three ways. The first and most certain is by grafting, i.e. by establishing organic union between the diseased and healthy tissue. There is a number of diseases in which no other artificial method has as yet succeeded, e.g. peach yellows, a disease of peach trees which is frequently fatal. Some other mode of transmission must, however, exist, since the diseases spread from plant to plant in the field. The second is by means of insects, which are fed on the diseased plant and then transferred to the healthy plant. There is a group of diseases in which this is the only method known other than grafting, e.g. leaf-roll of potato. The third is by inoculation of expressed juice or crushed tissue from the infected plant. This is fairly common, especially in the mosaic group, and most of the characters attributed to plant viruses have been worked out in diseases where it is possible, because of the ease with which the infective material can be examined. In some cases it succeeds with remarkable facility. In tobacco mosaic, for example, it is enough to rub a leaf of an infected plant between the finger and thumb and then rub a leaf of a healthy plant with the same fingers. But in other cases, e.g. mosaic of sugar-cane and curly-top of beets, infection can be thus produced only with the greatest difficulty, and in a very small percentage of plants. The difficulty may be partly technical, and the number of cases where diseases cannot be transmitted except by insects or grafting is gradually diminishing with improvements and modification of technique. But technical difficulties cannot be the whole explanation. K. M. Smith (1929[1]) infected a potato with both leaf-roll and mosaic, and fed aphids of the species *Myzus persicæ* Sulz. upon the infected plant. One sample of the aphids was then transferred to normal potato, and to it they transmitted leaf-roll but not the mosaic, although the potato was of a variety highly susceptible to mosaic. Another sample of the aphids was transferred to normal tobacco, and transmitted to it the mosaic, but not the leaf-roll (which is not transmissible to tobacco at all). The aphids, then, had taken up the mosaic as well as the leaf-roll from the infected potato and presumably injected it with the leaf-roll into the normal potato, but mosaic infection did not follow. A proper elucidation of the failure to obtain infection in such cases would be a fruitful contribution to our knowledge of plant viruses.

In diseases like tobacco mosaic, where inoculation of very small quantities of juice is enough to convey infection, an insect might be regarded as merely a mechanical carrier of the virus from one plant to another ; and possibly this may be the case where one insect is able to transmit several different diseases, e.g. *Myzus persicæ*, which can carry the mosaic of spinach, tobacco, lettuce and sugar-beet, as well as leaf-roll of potato. But in the numerous cases where artificial injection of plant extract is rarely or never successful, mere mechanical carriage would seem to be excluded, and the insect itself must influence in some way the infectivity of the virus. In such cases several insects may possess the power of conveying infection, or only one or two. In curly-top of beets at least nine different insects commonly found on beets or related plants have been found not to transmit (Stahl and Carsner, 1923) and the disease is carried only by a jassid, *Eutettix tenella*, or in the Argentine (where *Eutettix* does not occur) by *Agallia sticticollis* Stål (Fawcett, 1927). The mosaic of beets, on the other hand, a different disease, is carried by *Myzus persicæ* but not by *Eutettix*. In aster yellows the disease is transmitted by *Cicadula sexnotata*, also a jassid, but not by any of eight other insects which have been tried. *Amphorophora sensoriata*, one of the commonest aphids on raspberries, transmits none of the several virus diseases to which this plant is liable, while *Amphorophora rubi* transmits three varieties of mosaic but not curl, and *Aphis rubiphila* transmits curl readily, but rarely, if ever, any of the others (Bennett, 1927).

There is then some degree of specific relationship between certain viruses and their vectors, but its nature is still quite unknown in most instances. Four cases, however, have been reported in which it has been found that a definite interval of time must elapse after the insect has fed for the first time on a diseased plant, before it is able to transmit the disease. *Eutettix*, kept at ordinary temperatures, will not transmit the disease till at least 10 hours after feeding on a diseased plant, and a higher percentage of success is got after 24 to 72 hours. If the insect is kept at about 38° C. it can transmit in 4 to 6 hours but not sooner (Severin, 1921 ; Smith and Boncquet, 1915). In streak of maize a minimum of 6 to 12 hours must elapse at 30° C. before the leaf-hopper *Balclutha mbila* can transmit (Storey, 1926). In leaf-roll of potato, it is said that an incubation of 24 to 48 hours is necessary before the aphid becomes infective (Elze, 1927). It is to be remembered that if mechanical external carriage is excluded, the virus must pass from the alimentary canal of the insect to the salivary glands before it can be injected, and we do not know how long the process may require. In the fourth case, however, a much longer incubation period is required. *Cicadula sexnotata* transmits regularly the yellows disease of asters, but the adult does not become infective till at least six, and usually ten, days after feeding, and the nymphs also require a long period amounting to several days (Kunkel, 1926). These instances suggest that the virus requires to go through some developmental or other stage within the insect before it becomes fully infective, and, if so, a specific relationship is readily intelligible.

Further evidence of such relationship is given by the persistence of infectivity within the insect. *Eutettix* may remain infective for 111 days, i.e. its whole adult life (Severin, 1924). An individual *Balclutha mbila* retained the virus of maize streak for four months, during which it was fed only on uninfected sugar-cane (Storey, 1926), and *Cicadula sexnotata,* fed on rye plants, which are immune to the virus of aster yellows, still produced infection in asters after two months (Kunkel, 1926). The aphis *Macrosiphon solanifolii* transmitted the virus of spinach-blight, a mosaic, to the fourth generation of parthogenetically produced offspring (McClintock and Smith, 1918). This last case suggests that multiplication can occur within a suitable insect, and by the indication that it is not confined to the presence of living cells of particular plants encourages the hope, as yet unrealized, that some method of cultivation of virus *in vitro* may eventually be discovered.

Number and Specificity of Viruses.

Under standard conditions of host plant and environment a virus disease regularly comes true to type. There is a range of variation in the symptoms in different individuals, but the general picture is consistent. When a host is subject to several distinct diseases, each on transfer reproduces itself only, and does not give rise to any of the other types. The curly-top of beets does not produce beet mosaic, nor the mosaic of potato produce leaf-roll. In such cases one can hardly doubt that the viruses causing these distinct diseases are themselves distinct, and it seems certain that there are several different plant-viruses. How many there may be it is impossible to say. At least five, perhaps seven, different virus diseases occur in potato (Schultz and Folsom, 1923), and eight types of mosaic disease have been recognized in tobacco (Johnson, 1927). It is possible that some of these have arisen through modifications of a single virus. It is known that such modifications can occur. Passage of the virus of curly-top of beet through certain other species of plants, e.g. *Chenopodium murale,* attenuates it, apparently permanently, so that on return to the beet it thenceforth produces only a mild form of the disease (Carsner, 1925). Attenuation of the virus of tobacco mosaic by the action of heat is reported (Johnson, 1926[1]), and an increase of the virulence of potato mosaic on repeated passage through tobacco or tomato has been recorded by more than one observer (Johnson, 1925; Smith, K, 1929[2]; Henderson Smith, 1928[2]). Many of the diseases included in the large group of the mosaics may be due to strains of a single virus specialized by contact with different hosts. There is as yet, however, no established instance of the conversion of one virus disease into another.

Not only may one host be subject to several viruses, but one virus may attack several hosts, and in well-investigated diseases such as the mosaic of tobacco, sugar-cane and cucumber, long lists are known of different plants which are susceptible to the respective viruses. There is frequently some degree of specificity in the host-virus relation. The mosaic, for example, of tobacco, a solanaceous plant, is readily transmissible to at least twenty-five other Solanaceæ, but has not yet been certainly conveyed to plants belonging to any other family. Even this limited specificity, however, is not found universally. Cucumber mosaic is transmissible not only to other cucurbits but to plants of several other families (Johnson, 1927 ; Hoggan, 1927), and cross-inoculation between families is recorded in a number of other instances (Elmer, 1925 ; Fernow, 1925 ; Kunkel, 1928). Much of the evidence on this subject requires confirmation and more careful investigation with viruses of established pedigree and known purity.

A satisfactory method of identifying and classifying the viruses of plants has not yet been found, and is very much needed. At present we are mainly dependent upon symptomatology, and this, especially among the mosaics, is unsatisfactory, not only because the symptoms are themselves, as already pointed out, dependent on the environment, the condition and the variety of the host, but also because they are in many cases difficult to distinguish even on the same host. Something may be accomplished by the use of differential hosts, e.g. cucumber mosaic is transmissible to *Phytolacca decandra*, tobacco mosaic is not (Johnson, 1927) ; potato mosaic is transmissible to *Solanum dulcamara*, yellow tomato mosaic is not (Henderson Smith, 1928^2). In those cases where the disease can be conveyed by extracted juice, differentiation is occasionally possible by study of the characters of the virus *in vitro*. Perhaps use might be made of the specific virus-insect relationship. But all such methods are laborious and applicable in only a limited number of cases.

Characters.

Considerable attention has been paid to the properties of viruses as contained in the juices expressed from infected tissue after grinding. Tobacco mosaic may be taken as an example which has been very fully examined, but other viruses differ from it in many respects. Tobacco virus is readily filterable through the finer Berkefeld and Chamberland candles, and will pass collodion membranes up to a definite grade of porosity, which varies with the acidity of the juice (Mulvania, 1926). It remains infective on simple storage for at least five years (Dickson, 1925). It withstands drying, whether in air or over sulphuric acid (Allard, 1916), and dried tobacco leaf has been found infective after 31 years (Valleau and Johnson, 1927). It is not destroyed by heat up to 80° C.; even two days at this temperature failed in one case to abolish its infectivity (Mulvania, 1926); but it is usually inactivated in 10 minutes between 85 and 90°. It is very resistant to sunlight and withstands X-rays for half an hour, but is destroyed in one hour by rays from a mercury-vapour lamp (Mulvania, 1926). It withstands acetone (Vinson, 1927) and alcohol up to 90 per cent. for an hour, the virus coming down with the precipitate (Walker, 1926), and is also brought down by saturation with ammonium sulphate (Vinson, 1927). It is not destroyed by glycerin (Klebahn, 1928), toluene or chloroform and is resistant to most antiseptics (Allard, 1916). The juice remains infective after dilution to 1 in 10^4 with water, and, exceptionally, even when diluted 1 in 10^6 (Allard, 1915). This virus, then, is highly resistant, and the mosaics of tomato and ground-cherry, and the yellow or aucuba mosaic of tobacco and tomato have similar characters, so far as they have been tested (Walker, 1926; Henderson Smith, 1928[1]). In most of this work the results obtained have been purely qualitative, consisting only in a decision as to whether or not the juice was still infective after treatment. It is only recently that quantitative methods of examination have been introduced (Holmes, 1928[2]; McKinney, 1927[2]). When these are more fully developed, we shall get a deeper insight into the changes that the virus may undergo, e.g. it is found that juice on simple storage increases in infectivity at first and then begins slowly to deteriorate (McKinney, 1927[1]; Holmes, 1928[2]).

Potato mosaic is distinctly less resistant than tobacco mosaic. It is filterable through the Chamberland L3 filter, but is destroyed in 10 minutes at 80° C., and by 90 per cent. alcohol in an hour (Henderson Smith, 1928[2]), and some strains are even less stable (Johnson, 1925). Cucumber mosaic is filterable through Berkefeld but not through Chamberland candles, loses its activity in a week on standing, and does not withstand drying, precipitation by even 45 per cent. alcohol, heating over 70° C., or any antiseptics tried except toluene and 5 per cent. chloroform (Doolittle, 1920). The conclusion was drawn from these differences that the virus of cucumber mosaic must be different from the virus of tobacco mosaic. It has to be remembered, however, that tests of this kind are carried out, not with isolated virus, but on virus contained in the juice of the host plant; and Walker (1926) has maintained that when cucumber mosaic was transferred to ground-cherry, and its properties examined in the juice of the new host, it had lost its labile character, and conversely that ground-cherry mosaic transferred to cucumber acquired the labile characters of cucumber mosaic. The apparent differences in resistance, therefore, are, according to Walker, due to differences in the juices of the hosts, and not to a real difference in the virus themselves. Walker's experiments, however, are not wholly convincing, and it cannot be taken as established that cucumber mosaic does in fact lose its labile character on transference to another host (Johnson, 1927).

Ætiology.

The fact that they are retained by filters of a sufficient grade of fineness has led to the general belief that plant-viruses are particulate, and most workers incline to the view that they are also living parasites, while recognizing that the evidence is not conclusive. The size of the virus of tobacco mosaic is put at about $30\mu\mu$, again on the evidence, for what it may be worth, of filtration experiments (Duggar and Karrer, 1921).

No visible organism has yet been isolated with a substantial claim to be the cause of any plant virus disease. From time to time accounts have been published of bacteria or mycetozoa or protozoa, usually flagellates, said to occur only in plants infected with virus (Iwanowski, 1903; Eckerson, 1926; Jones, 1926; Paine and Bewley, 1919). Some of these are of comparatively large size, but this is not necessarily a valid argument against their acceptance, since their life-cycle may include a filterable or ultra-microscopic stage. In no case, however, has causal connection been satisfactorily proved.

Multiplication *in vitro* has also not been certainly obtained. Olitsky (1925) states that he has obtained infection with the twelfth subculture in sterilized tomato juice of the virus of tomato mosaic; but other workers (Mulvania, 1925; Purdy, 1926; Goldsworthy, 1926; Henderson Smith, 1928[1]) have failed to confirm his results, and up to the present no plant virus has been proved to multiply outside a living host.

Intracellular bodies, on the other hand, occur regularly in many virus diseases, and can easily be seen in the living unstained cell. They were first described by Iwanowski in 1903 in tobacco mosaic, and since then have been reported in a considerable number of diseases in plants so diverse as, e.g. Graminaceæ (Kunkel, 1921; McKinney, Eckerson and Webb, 1923), Amaryllidaceæ (Kunkel, 1924; Holmes, 1928) and Solanaceæ (Goldstein, 1926; Dickson, 1922; Sorokin, 1927; Klebahn, 1928). They differ somewhat in different diseases and in different hosts, but are sufficiently alike in most of them to make it probable that they are structures of similar nature. They are individual masses of material, usually vacuolate, which has the appearance and staining reactions of protoplasm. They are frequently roughly spherical, with sometimes a definite indication of a bounding membrane, but may be of different shapes even in the same tissue, varying from bodies nearly round to elongated bolster-like structures, which are sometimes constricted in the middle, and may be of quite irregular outline. In size the spherical bodies range from 3 to 4μ or less up to 35μ in diameter, and the elongated forms may in certain hosts, e.g. maize, hippeastrum, be considerably larger. Independent motion has not been observed, but they may be carried with the protoplasmic streaming from place to place within the cell. They tend to be closely associated with the nucleus, often lying up against it or moulded round it, but are not incorporated with it, and may be separated from it by a considerable interval without any apparent connection with it. They occur almost exclusively in areas where other signs of disease are perceptible, and are found close to the growing points and in the stem, flower and root as well as in most of the leaf tissues, including the phloem tubes and phloem parenchyma.

It is certain that these x-bodies, as they have been well named by Miss Goldstein, are associated with the virus infection. They do not occur in normal plants, nor in plants suffering from other forms of disease, nor in the non-infectious chloroses of variegated plants (Smith, F., 1926). Their occurrence appears to depend more on the nature of the particular virus than on the nature of the host, since one virus, e.g. tobacco mosaic, produces them in a large number of hosts, while another virus, e.g. cucumber mosaic or potato mosaic, may fail to produce them in the same hosts even when the symptoms are equally conspicuous in both cases (Hoggan, 1927).

What their nature is, is still uncertain. Most workers regard them as products of the cell itself, an effect of the metabolic disorder produced by the infection. Others believe that they are alive and may be a form of the parasite itself. Their resemblance to amœbæ was noted by Iwanowski, and the occurrence in plant tissues of well-known parasites not very dissimilar is familiar. They increase in size, and two or more may be found in a single cell. Goldstein believes that they divide by constriction, and are distributed by the division of the cells of the host. The evidence that they are actually living entities is, however, still inconclusive. They sometimes contain deeply staining granules, and apparently contain chondriosomes (Holmes, 1928[1]), but nuclei have not been demonstrated in them.

In addition to the x-bodies other intracellular structures are regularly met with, and only in infected plants, e.g. striate masses, threads, and bundles of crystal-like material ; but to none of these can any ætiological significance be attached.

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CHAPTER IV. VIRUS DISEASES OF INSECTS.

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Introduction.

THE virus diseases of insects may be divided into two classes—the first comprising 'sacbrood' of honey-bees (*Apis mellifica*), in which inclusion bodies are not present, and a second group in which nuclear inclusions can be demonstrated. This section includes the polyhedral diseases of the gipsy- and nun-moth caterpillars, the condition described as 'grasserie' of the silkworm as well as similar infections met with in other lepidopterous larvæ, which have not been the subject of such close study.

Up to the early years of the present century the virus diseases were confused with bacterial and protozoal infections, the results published by v. Prowazek (1913) on his researches with grasserie material from silkworms being the first demonstration of virus infectivity with bacteria-free Berkefeld filtrates. The filterable virus of sacbrood was demonstrated by White (1913), and the same year Glaser and Chapman (1913) described a virus infection of the gipsy-moth larva. The filterability of the virus of 'Wipfelkrankheit', a disease of the nun-moth caterpillar, was first described by Komárek and Breindl (1924), and two new virus diseases of the caterpillar of the cabbage butterfly (*Pieris brassicæ*), in which both nuclear and cytoplasmic changes occur, were subsequently investigated by Paillot (1926).

Apart from the desirability of the study of these conditions as part of the general investigation of virus diseases, the economic aspect of the subject is of practical interest. In the case of sacbrood of bees and grasserie of silkworms the elucidation of problems of control in limiting the ravages of local and recurrent infections is of considerable economic importance. In the case of virus diseases of the lepidopterous larvæ, on the other hand, the best means of promoting and assisting the spread of natural infection must be determined.

Sacbrood of the Honey-bee.

History. This condition, as distinct from American and European foulbrood (see Vol. III), has been recognized by bee-keepers for many years. But our present knowledge of the ætiology and nature of the malady is due to the careful and extensive studies of the American investigator White (1917), who first gave the name 'sacbrood' to the disease owing to the fact that many of the dead larvæ can be removed from the cell without rupturing their body walls, and, when thus removed, have the appearance of a small enclosed sac.

Symptoms. The strength of the hive colony is often not diminished to any great extent, unless the comb is badly infected. The brood die after the time of capping within capped cells during the last days of the larval stage. The dead larvæ are, therefore, posturally in the last stage of their existence, i.e. extended lengthways in the cell and lying with the dorsal side against the lower wall. It is unusual to find larvæ dead in uncapped cells, but the cappings are frequently punctured by the adult insects. The dead larvæ lose their normal colour and assume a slightly yellowish tint, the form of the larvæ changing very much less than in foulbrood. The contents of the sac-like larva, at first more or less watery, gradually evaporate and the whole organism is reduced to a dry mummified mass, known as the ' scale '.

The condition may be differentiated from American foulbrood by the absence of odour from the brood combs; the ' ropiness ' of foulbrood is also not present and the exciting cause of American foulbrood (*B. larvæ*) cannot be demonstrated. In European foulbrood death takes place in uncapped cells, the larvæ being usually coiled in the cell before the endwise position is assumed. The causal organism of this condition (*B. pluton*) is not present in sacbrood. Adult bees are not susceptible to sacbrood, which is an infectious, albeit benign, disease of the brood.

Cause of sacbrood. The ætiological factor is a filterable virus. The virus contained in a single larva recently dead of the disease is sufficient to produce infection and death in at least 3,000 larvæ within a week, the sacbrood virus being capable of increasing with enormous rapidity. The virus is killed by heating in water at 60° C. for ten minutes. In honey, however, a temperature of 70 to 73° C. is needed for the same period, but when heat is not applied and the virus is shaded from direct sunlight it remains alive for nearly a month. Dried virus exposed to the direct rays of the sun is destroyed in from four to seven hours.

Methods of infection. It has been shown experimentally that when larvæ, sick or dead from the disease, are picked from the combs, crushed and diluted with sterile water and the suspension subjected to Berkefeld filtration, the filtrate so obtained is capable of infecting healthy larvæ. In nature the virus is transmitted from diseased to healthy larvæ within the colony, or by a diseased colony to a healthy one. The incubation period is six days, or slightly less. Histological investigation does not reveal the presence of cellular inclusions or reaction bodies, such as are met with in the virus diseases of lepidopterous larvæ. The contents of the dead larvæ have a granular appearance, due to the presence of numerous fat-cells of irregular shape, containing black-staining spherical bodies. The moult skin is separated from the hypodermis by a watery coagulable fluid.

Under natural conditions the disease is more often encountered during the first half of the brood-raising season than during the second half. The dead or diseased larvæ are generally removed by the adult bees, the workers possibly feeding the pieces of infected tissue to the adults and older larvæ, but not to the young and highly susceptible grubs. The general tendency is towards recovery in the colony. There appears to be little possibility of the virus being transmitted by way of flowers visited by bees—the water supply of the insects being the probable source of infection.

Control. The infected combs should be stored for one or two months before they are again used, as drying appears to destroy the virus within that time. Very little is known concerning treatment and prophylaxis of sacbrood. There does not appear to be any fear of the disease being transmitted by hands or clothing or by tools used about the apiary. Flaming the hives or disinfection of the surrounding ground does not appear to be necessary.

The Polyhedral Diseases: Wilt Diseases of Nun- and Gipsy-moth Caterpillars and Jaundice of Silkworm.

The polyhedral affections of insects attack only the larval and pupal stages of certain lepidoptera, and in so far as they are incident to agricultural and forest pests (gipsy- and nun-moth larvæ) contribute substantially towards their natural control. The conditions met with in America, including the polyhedral diseases of the larvæ of the gipsy-moth (*Porthetria dispar*), tent caterpillar (*Malacosoma americana*), army worm (*Leucania unipuncta*), and other species are described as ' wilt ', while in Europe a condition, similar to or identical with gipsy-moth infection, present in the nun-moth caterpillar (*Lymantria monacha*) is called 'Wipfelkrankheit'. When appearing in the silkworm (*Sericaria mori*) the condition is called ' grasserie ', ' jaundice ', ' Gelbsucht ' or ' Fettsucht '.

Symptoms. The conditions met with in the silkworm and the larvæ of the nun- and gipsy-moth—the three virus diseases that have been most carefully studied—are essentially the same. In the case of grasserie of the silkworm the affected areas are usually covered with lemon-yellow blotches, and a few days before death the larvæ become inactive and refuse food. The skin assumes an opaque shiny yellow appearance before death. The nun-moth shows few recognizable symptoms except loss of appetite and a tendency to migrate to the tops of the trees (' Wipfel ') where, hanging by the prolegs, they die in large numbers. The symptoms of wilt disease of the gipsy-moth are substantially the same. The caterpillars stop eating, become languid, and usually crawl up to some object, where they remain motionless. In a few hours a dirty blackish, foul-smelling liquid drops from the mouth and anus, they become more and more flaccid, the legs lose their support and the caterpillars are reduced to a black skin hanging limply to the tree trunks and branches. In the silkworm the blood has a cloudy, yellowish white appearance : in other caterpillars, a cloudy brownish colour.

Polyhedral bodies. Within a few hours of death all the tissues are disorganized, and microscopical examination of fluid from dead caterpillars shows that it contains the elements of broken-down tissues, and myriads of polyhedral bodies of various sizes. In the silkworm the average polyhedron is 3 to 5μ in diameter, the individual being found as small as 0·5μ and as large as 15μ. The average polyhedron of the gipsy-moth measures 3·4μ in diameter, that of the nun-moth 2·65μ. They occur singly or in pairs, are very refractive and superficially resemble crystals. The shape varies (in silkworms there are up to eight facets or more), and the corners are often sharp or angular. On examination the bodies appear to have a dense centre differentiated from a somewhat lighter peripheral mass. Growth proceeds by accretion. The polyhedra crack readily into a number of pieces when pressure is applied. Nothing has been observed to escape from these bodies, which have, during the progress of fragmentation, often been confused with fat bodies and urate crystals. The polyhedra, however, do not stain with Sudan III, or blacken with osmic acid, and are not soluble in ether. They are very resistant towards stains in general, and unless the stain has been applied for a long time and is followed by a mordant, they only take the colour round the periphery.

The dark field reveals in the fluid from dead caterpillars, small, dancing, refractive granules (Brownian movement), which, however, have no ætiological significance. They are heavier than water and can be obtained in bulk by centrifuging aqueous emulsions of diseased material. They do not dissolve in hot or cold water, carbon bisulphide, alcohol, ether, chloroform, &c., but are soluble when heated in strong solutions of acids and weak alkalis. Picric acid stains them yellow, showing that they contain protein material. They may be regarded as crystal-like degeneration products of nucleoprotein origin.

Predisposing and exciting causes. All the larval stages are susceptible to polyhedral disease, but it is less easily transmitted to the young than to the old larvæ. Young pupæ acquire the disease readily, but after the adult moth tissues are formed it appears impossible to induce infection. The incubation period is appreciably shortened by exposing the animals to high temperatures. In nature, infection takes place through the mouth by means of the food. Lack of food, with consequent loss of vitality, produces a greater susceptibility to the disease. Climatic and weather conditions are of less significance than in other caterpillar diseases.

Silkworms fed with infected material assume a jaundiced appearance in from 4 days to 1 week, and death generally occurs in from 5 to 10 days. Infection can be transmitted indefinitely from one series to another. With gipsy-moth larvæ the time from infection by feeding to death is somewhat longer, on an average 21 days. Infectious blood greatly diluted (1 : 1,000,000) still retains its infectivity and is capable of infecting healthy insects. The virus in diluted blood passes through Berkefeld filters, being filterable through Berkefeld V and N candles. It can be filtered at a pressure of 74 mm. through Berkefeld W candles with difficulty, but cannot be passed through Pasteur-Chamberland filters

(Chapman and Glaser, 1915). These results probably depend on the size or formation of the filter pores, upon the flexibility or rigidity of the virus particles and other factors.

The polyhedral bodies can be freed from active virus by repeated washing and centrifuging. The bodies so treated, entire or fragmented, are incapable of producing disease when injected into larvæ or fed to them. However, Aoki and Chigasaki (1921) have attempted to prove that the polyhedra are parasitic and Komárek and Breindl (1924) claim that the virus resides within the polyhedral bodies.

Host specificity of virus. The various virus diseases of caterpillars, though similar histo-pathologically, are nevertheless caused by different infecting agents, it having been shown by Glaser (1928) that silkworm grasserie, tent-wilt and gipsy-wilt virus are distinct, and specifically limited to their hosts. A series of experiments was carried out, both by inoculation and by feeding healthy gipsy-moth larvæ with infectious tent-wilt blood, and a similar experiment was carried out with infectious silkworm grasserie blood. No disease resulted. Similarly, tent caterpillars were found to be insusceptible to gipsy-wilt and silkworm grasserie, and silkworms to gipsy- and tent-wilt. Chapman and Glaser (1916) also inoculated gipsy-moth larvæ with sacbrood virus ; the result was negative as was also that of the reciprocal experiment with bee larvæ.

Immunity and transmission of virus through ova. It has been observed in severe epizootics of wilt and grasserie that certain individual larvæ, though infected, do not develop polyhedral disease, and transform into adults : thus a complete extermination is never possible owing to the immunity of certain individuals in each generation. There is no direct evidence, however, that infection can be transmitted through the egg to the succeeding generation, though Chapman and Glaser have adduced indirect evidence of such transmission. The eggs from tent-moths that survived experimental larval infection with tent-wilt virus were separated and hatched out with all possible precautions against extraneous contamination. But in spite of such precautions, 12 per cent. of the hatched-out larvæ died of wilt in the fourth or fifth instar. It thus seems likely that the polyhedral diseases are transmitted from generation to generation through the eggs of a certain proportion of individuals.

Resistance of virus to physical and chemical agents. Grasserie or wilt blood when kept at 60° C. for 15 to 20 minutes loses its virulence, but when dried at room temperature remains infective for at least two years. The virus of Wipfelkrankheit is virulent under the same conditions after three years, and will resist glycerol for five days. Wilt and grasserie blood retain their virulence in 98 per cent. glycerol for six months, and wilt and Wipfelkrankheit virus withstand putrefaction. These viruses are inactivated by exposure for nine hours to sunlight. Wilt blood dried and submerged in 5 per cent. phenol becomes inactive in approximately three weeks.

Control. The polyhedral viruses of insects are extremely infectious, healthy larvæ being readily infected by food contaminated with the tissues or fæces of infected cadavers. When polyhedral disease is suspected, or appears in experimental stock, the whole should be burned or otherwise destroyed. Eggs from infected moths that have recovered from disease should never be bred for hatching purposes. Foreign eggs of unknown purity should never be employed, and care should be taken not to introduce infection from contaminated foodstuffs.

The spread of polyhedral disease among larvæ may possibly be assisted by spraying with emulsions of cadavers from the previous year; but no unequivocal conclusion can be drawn from such field experiments as have, up to the present, been carried out.

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CHAPTER V. FOOT-AND-MOUTH DISEASE.*

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Clinical Description.

FOOT-AND-MOUTH disease (Maul- und Klauenseuche, fièvre aphteuse) is a highly contagious infection caused by a filterable virus which occurs chiefly in cattle, pigs and sheep. For purposes of description, animals may be grouped into (*a*) those experimentally susceptible, (*b*) those naturally susceptible, (*c*) those doubtfully susceptible or resistant.

Experimentally susceptible animals.

Guinea-pigs. Hecker (1899) claimed to have infected guinea-pigs with foot-and-mouth disease, but it was not until Waldmann and Pape (1920, 1921) published the details of inoculations in series that the importance of this animal for research in this field was realized. Hobmaier (1921) and many others since have amply confirmed their results. Gins and Fortner (1924) produced infection through inhalation of fine particles of the dried contents of vesicles.

The lesions in the guinea-pig occur on the pads of the fore and hind feet, the tongue and mucous membrane of the lips. When the plantar skin has been inoculated a primary vesicle appears in from 18 hours to 7 days. An incubation period of more than 36 to 48 hours is uncommon unless the virus is weak or not adapted to the guinea-pig. Healing is progressive, usually without secondary infection. Complete restoration requires about 3 weeks. At the time of appearance of the primary vesicle virus is present in the blood, and it can be detected there until secondary lesions develop. The interval varies from about 18 hours to 3 days. Animals weighing about 350 gm. which are gaining in weight are most susceptible. Young animals or older ones in poor general condition do not react well. Larger doses of virus are required to infect by any route other than inoculation of the plantar skin. No evidence has been obtained that the virus multiplies elsewhere than at the site of the lesions. The mortality in guinea-pigs is about 5 per cent., and nearly all the deaths appear to be attributable to the lighting up of latent infections. In guinea-pigs the infection is not communicable. Why this should be so has not been explained, but the fact is probably of fundamental importance.

That pressure or the indirect result of pressure is one factor which determines the multiplication of the virus and development of lesions in certain tissues of the guinea-pig has been demonstrated experimentally (M. C. Maitland, 1928).

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* For a comparison of foot-and-mouth disease and vesicular stomatitis Chapter VI should be consulted.

Rabbits. Foot-and-mouth disease in the rabbit has been investigated in the light of recent knowledge by Hobmaier (1921), Gins and Fortner (1926), Nicolau and Galloway (1925), Burbury (1927, 1928). All are agreed that the rabbit is susceptible to infection if inoculated on the mucous membrane of the tongue, gums or lips with guinea-pig vesicle fluid. It is possible to pass the infection through a series of rabbits by inoculating from tongue to tongue. Gins and Fortner recorded 20 passages, the other authors 13 and 8 passages. The virulence of the virus for guinea-pigs was not decreased by rabbit passage. Burbury failed to infect rabbits by feeding them with heavily infected bran. She recorded contact infection in only two animals, which had wounds, and stated (1928) that it was more difficult to infect rabbits with virus direct from cattle than with guinea-pig-adapted virus.

Rats. The disease in rats has been investigated by Beattie and Peden (1924), Beattie, Morcos and Peden (1928), and Burbury (1927, 1928). The first-named authors made experiments on rats as possible disseminators of infection and obtained what they considered to be suggestive, but not conclusive, evidence that they might be implicated. Burbury found that wild rats (*M. norvegicus*) were only slightly susceptible to guinea-pig virus and even less to cattle virus. The virulence of the virus for rats was enhanced by successive passage from foot to foot. Spontaneous infection was not observed, nor was infection produced by feeding guinea-pig epithelium from primary vesicles. White rats were less susceptible and not spontaneously susceptible. Fourteen successive passages were carried out from tongue to tongue, but the virus did not become increasingly virulent for the species.

Naturally susceptible animals.

Cattle. The incubation period of the naturally acquired infection is from 2 to 4 days, occasionally longer. The onset is marked by fever. Within 12 to 36 hours after fever begins, lesions develop on the tongue, lips, gums, hard palate and feet. The fever then decreases and usually after 24 to 36 hours the temperature is normal. As a rule there is no secondary rise. The vesicles soon rupture, leaving eroded patches. The situation of the earliest lesion is not uniform and one or more of the sites mentioned may not be affected. Lesions sometimes occur on the udder and teats. Salivation is a characteristic feature. Lameness is frequently noticed early. Recovery is the rule. Secondary infection sometimes occurs in the feet and in the teats, but usually healing is uncomplicated and completed in 10 to 14 days. The lesions of the feet in some cases persist for several weeks ; the horn of the hoof may be undermined and separate, or in healing a cavity may be enclosed in which virus persists. The severity of the disease varies considerably in different animals in the same herd.

A severe form of the disease is also recognized. In this form sudden deaths may occur after the period of fever when the lesions are beginning to heal. They have been associated with hæmorrhagic and degenerative changes in the myocardium. Lesions in the rumen may also be found.

Pigs, sheep and goats. The disease in these animals has the same general features as in cattle, but is usually less severe.

Marmots. Klobouk (1926) reported that the suslik or pouched marmot ' Zieselmaus ' was very susceptible to infection and that the virulence of the virus for guinea-pigs was not decreased by passage in this species.

Hedgehogs. Stockman and Minett (1927) infected two adult hedgehogs by inoculating them with guinea-pig virus.

Deer and reindeer. Magnusson (1928) demonstrated that reindeer were naturally susceptible and mentions an outbreak of foot-and-mouth disease among buffaloes and bisons in the zoological gardens in Copenhagen. Instances of the infection of deer are mentioned by Gins and Krause (1924).

Doubtfully susceptible and resistant animals.

The diagnosis of foot-and-mouth disease in animals whose susceptibility to this infection is doubtful can now be made with certainty by transferring the disease to guinea-pigs or other susceptible animals and carrying out cross-immunity tests. The need for experimental diagnosis applies equally to lesions observed in fowl and other birds and is particularly necessary whenever the lesions deviate from those of naturally susceptible species.

Mice. Arkwright and Burbury (1925) failed to infect white mice and house mice with guinea-pig virus. After the intramuscular inoculation of wood mice (*Apodemus sylvaticus*) the only lesions observed were small vesicles on the tongue in some animals. Direct contact did not lead to infection. Stockman and Minett (1927) failed to infect white mice.

Dogs and cats. Experimental infection of 32 dogs was attempted by Stockman and Minett (1927). In 3 only (1 adult and 2 seven-day puppies) were lesions observed. Contact infections were not obtained. They inoculated 10 adult cats and 10 kittens and in 6 there was evidence of infection. Contact infection did not occur. Hecker (1899) claimed to have infected dogs and cats. Loeffler and Frosch (1898) stated that they had observed natural infection in dogs, but were unable to infect dogs and cats by inoculation. Gins and Krause (1924), reviewing the scanty and somewhat contradictory earlier literature, state that the susceptibility of dogs and cats is very slight, an opinion supported by the experiments of Stockman and Minett.

Ferrets. Galloway and Nicolau (1928) found the ferret to be almost completely resistant. In only one animal was a small microscopic lesion of the tongue found.

Fowl. Stockman and Minett (1927) inoculated 34 fowls without producing any clinical effects. An additional 12 fowls were fed with virus and the fæces tested for the presence of virus. In 3 fowls only was it detected in samples passed between 10 and 26 hours after feeding. They also reported the unsuccessful inoculation of ducks.

Other birds. Wagener (1925) and Stockman and Minett (1927) have noted the insusceptibility of the sea-gull. The latter authors have failed to infect martins and sparrows.

Horse. The horse is not susceptible to foot-and-mouth virus (see Chapter VI).

Foot-and-mouth disease in man.

Although man is relatively insusceptible, human cases have occurred. Arkwright (1928) has summarized the subject and Gins and Krause (1924) and Cadiot (1914) have critically reviewed the earlier literature. Good clinical descriptions of undoubted cases are those recorded by Pape (1921) and Gerlach (1924). As far as records of undoubted cases permit a definite statement to be made, foot-and-mouth disease in man is characterized by an abrupt onset with fever, followed shortly by vesicular eruption on the mucous membrane of the lips and mouth, on the palms, soles, and about the nails. Vesicles may not appear in all of these situations. There may be salivation and diarrhœa. The fever lasts until the lesions have developed, a period of 2 to 4 days. The vesicles recede promptly and healing is rapid. A differential diagnosis cannot, however, be made with certainty on clinical grounds alone. All suspected cases should be confirmed by infecting a susceptible animal and demonstrating the nature of the virus by cross-immunity tests. Not all cases reported as foot-and-mouth disease have fulfilled these criteria ; the majority were recorded before the guinea-pig was recognized as an experimental animal in this connection. Many are, therefore, open to doubt, particularly those cases which had cutaneous lesions on areas other than the soles and palms or in which the lesions continued to develop for a prolonged period.

Foot-and-mouth disease in man is probably a comparatively rare occurrence. Transmission from man to man has not been demonstrated.

Epizootiology.

Foot-and-mouth disease is characterized by the appearance of the infection at irregular intervals in centres often widely separated, and by its rapid spread. The source of infection is usually obscure. The existence of three types of virus, producing the same kind of disease, but differing in that immunity to one does not always protect against the others, indicates that an immune population may not be readily built up by natural means. The possibility that animals may acquire increased resistance without obvious infection through contact in a locality where the disease is prevalent, has been discussed by Greenwood (1927).

Carriers.

Brandt (1928) has reviewed the literature and concluded that carriers among cattle were much less frequent than has generally been supposed. Assel (1913) and von Böhm (1913) found virus in the healed feet of cattle up to 8 weeks, and 251 days after infection. Schoening (1927) found no carriers in 20 recovered cattle. The hoofs of 22 cattle and 1 hog were also examined *post mortem,* 20 days to 6 months after experimental infection.

Only once, from a cow, 34 days after infection, was virus detected. Brandt (1928) stated that the evidence in favour of the hypothesis that virus can be conserved for long periods in the hoof is not free from doubt. He thought it probable that virus only persisted in the form of contaminated matter in the cracks of the feet. Waldmann and Reppin (1927) examined the persistence of virus in the saliva of experimentally infected cattle. It was present for 6 days after inoculation and regularly absent from that point on. If the saliva contained particles of epithelium from vesicles, virus was demonstrable for 11 days, 8 days after the appearance of mouth lesions, but not later. The virus was absent when the vesicle epithelium had been shed and the erosions were covered with healthy granulation tissue. The observations of Schoening (1927) were in agreement. Trautwein, Thomashoff and Höve (1928) have reviewed the somewhat contradictory literature on the presence of virus in fæces, urine, bile and milk, and have established that in cattle, swine and goats, it may be so excreted during a period which coincides with its presence in the blood. Lebailly (1926) has reported a similar relationship between excretion of virus and infectivity of the blood, and did not detect carriers.

Transmission of the disease from acutely infected animals.

Waldmann and Reppin (1927), Schoening (1927), Lebailly (1922 and 1926) and Vallée and Carré (1922[3]) agree that the saliva of cattle contains virus before lesions can be detected, and cattle are said to be highly infective at this stage. Waldmann and Reppin found virus in the saliva as early as nine hours after the inoculation which produced experimental infection. The exact mode of transference of the virus and the conditions requisite for its entrance into the tissues have not been fully demonstrated. The frequency of a mild type of infection which would escape detection in the field is difficult to ascertain, but possibly more importance should be placed on this aspect of the problem of how the disease is spread than has been customary in the past.

Transmission by spread of the virus through intermediate agents.

The survival of dried virus and its resistance to heat and cold are discussed in another section.

Carcasses. The survival of virus in infected carcasses has been investigated by Stockmann and Minett (1927). The muscle tissue of animals slaughtered when the blood is infective rapidly rids itself of virus owing to the acidity associated with *rigor mortis*. The bone-marrow of bacon carcasses dressed in the ordinary way and stored at 10 to 15° F. and at 28 to 30° F. was infective for at least 42 days and once after 76 days. Wet or dry salting did not alter the result. Lesions on the feet contained virus after 32 days' immersion in brine at 2 to 5° C. Blood from a heart stored at 10 to 15° F. was infective after 34 days. Similar results were obtained with beef carcasses.

Manure. Virus in stacked manure is destroyed in a few days according to Minett (1928) and Trautwein (1926[1]), in agreement with earlier observers. The heat generated naturally is an important factor in its destruction. Wagener (1928) cites instances which suggest, however, that some outbreaks may have originated from infected manure. One stack left in the open contained virus at the surface for 39 days. He emphasized the importance of an alkaline reaction due to ammonia generated in decomposing urine as a destructive factor and showed that manure collected and stored with the effluent in a tank was rapidly sterilized.

Sewage. Wagener (1927) noted that the survival of virus in sewage was influenced by temperature, and activity of decomposition. The latter appeared to impair survival. Acidity or alkalinity was also deleterious. In summer (17 to 21° C.) the virus survived for 14 to 21 days; in September and October (13 to 18° C.) for 43 to 49 days and in winter (4 to 13° C.) for 103 to 140 days.

Stables. Lebailly (1922) stated that susceptible cattle could be put in a stall in contact with infected cattle, after four days had elapsed from the rupture of vesicles in the mouth, without their contracting the disease. Vallée and Carré (1922[3]) agreed with this observation and noted the rapid loss of infectivity of dung and stables without disinfection.

Transmission by flies, insects and wild animals.

The experiments of Lebailly (1924) and the investigations of Kunike (1927) appear to have ruled out the possibility of transference of infection by the common fly and by *Stomoxys calcitrans.* Arkwright and Burbury (1925) were not able to transfer infection in guinea-pigs by bugs (*Cimex*). Lebailly (1925) recorded outbreaks which seemed to suggest an origin from wild animals and it is possible that further investigation would implicate certain species as potential sources of infection. Transmission by the migration of birds has been suggested by Stockman (1925) among others, but there is no satisfactory evidence in support of it.

Transmission by man.

Kling and Höjer (1926) advanced the supposition that man was susceptible to mild infection and was often a true carrier, harbouring virus in the mouth. Waldmann and Trautwein (1928[1]), reviewing the subject, were unable to confirm the existence of human carriers by direct experiment and hold that the theory of Kling and Höjer is very probably incorrect. In this opinion the majority will agree. Kling and Höjer (1928) attempted to confirm their hypothesis, but were unable to demonstrate virus in the nasopharyngeal secretions or fæces of humans. There is no adequate proof that man is susceptible to mild disease or can be a carrier.

Pathological Anatomy and Histology.

Foot-and-mouth disease is characterized by the formation of vesicles in epithelial tissues in various parts of the body. The earliest histological lesion consists in degeneration and necrosis of small foci of epithelial cells, comprising at first only a few cells. The protoplasm is ballooned, the nuclei are swollen or pyknotic. Polymorphonuclear leucocytes are present in small numbers in the earliest lesions. The process of epithelial necrosis spreads laterally and to some extent throughout the thickness of the epithelium, associated with larger numbers of polymorphonuclear leucocytes. As the lesion advances polymorphonuclear infiltration and increased vascularity become prominent in the subepithelial tissue. In this area there is also endothelial proliferation in varying degrees. Separation of the epithelial layer gives rise to a small cavity, an early vesicle, which when fully developed is covered by the stratum lucidum and a thin layer of underlying cells, and has for its base isolated areas of the stratum germinativum. The margins of the lesions show a rather sharp transition to normal tissue. The covering usually ruptures and restoration proceeds by the proliferation of the islands of epithelial cells left in the base of the lesion.

In the feet of cattle large areas of separation of the horn may be indicated by only a small crack and it is possible for vesicles to form and remain closed. In these the virus can persist for considerable periods after the feet are apparently healed ; as long as 251 days has been recorded.

Erosions and ulcerations of the stomach and intestine of cattle have been described, but their histology has not been studied so extensively as that of the epithelial lesions. Hæmorrhage into the mucous membrane of these parts has also been noted.

The earlier literature on these points has been well reviewed by Gins and Krause (1924). More recent investigations are those of Levaditi, Alberca-Laurente and Galloway (1926), Alberca-Laurente (1927), and Galloway and Nicolau (1928).

The ætiological relationship of the virus to the lesions described above is apparently well founded, but there is room for doubting if the recorded descriptions of round-cell infiltration in foci of degeneration and necrosis of the myocardium are lesions due to this virus, since the earliest and predominating leucocytic reaction in epithelial lesions is infiltration of polymorphonuclear leucocytes. It is generally accepted that secondary infection of the ulcers left by broken vesicles occurs : the development of membranes and necrosis of bone, for example, can reasonably be attributed to this factor. It would appear likely that catarrhal inflammation of the milk ducts, abscesses of the udder, enlargement of the spleen, and hæmorrhage into the serous membranes might also be found to be associated with secondary infection if the possibility were carefully investigated. The so-called malignant type of disease, which gives rise to cases of sudden death associated with marked changes in the myocardium, might be due to a generalized secondary infection.

The literature on the presence of inclusion bodies in this disease has been reviewed by Gins and Krause (1924) and by Gins (1922) in the article in which he described small bodies in the epithelial cells of guinea-pigs at the site of lesions caused by the virus. He did not believe that they represented the virus itself, nor did he conclude that they were specific for this disease. Trautwein (1925) found similar bodies not only in the epithelium of infected guinea-pigs but also in lesions produced by heat or acid, and in the stomach, duodenum, pancreas and spleen. His conclusion that they were not specific appears to be justified.

Levaditi, Nicolau and Galloway (1926[2]) and Galloway and Nicolau (1928) have stated that, in their terminology, the virus is an 'ectodermose pure'. It has long been recognized that lesions of the disease occur mostly in epithelial tissues, but only in certain areas. Other areas of epithelium and other tissues derived from ectoderm are not affected.

The Nature and Properties of the Virus.

The potency of the virus employed for experiments on its nature and properties is of primary importance, and one of the chief advantages arising from the use of the guinea-pig is that titration can easily be made.

Filterability.

It is of interest that foot-and-mouth disease was the first human or animal disease which was shown to be caused by a filterable virus (Loeffler and Frosch, 1898).

Filtration in relation to the porosity of candles has been studied by Olitsky and Boëz (1927[2]). The virus passed readily through Berkefeld V and N candles and through Chamberland filters down to L5. Through candles of finer porosity filtration was irregular: virus passed through 1 of 5 L7 candles, through 1 of 3 L9 candles and failed to pass any of 7 L11 candles. Some virus is always retained by the filters. Burbury (personal communication) has found that the first c.cm. of filtrate contains relatively little virus and that the maximum potency of the filtrate may not be reached until 20 to 30 c.cm. has been filtered through a small Seitz filter disc. In filtering about 40 c.cm. the titre may be reduced approximately from 1 in 5,000,000 to 1 in 500,000. The same authors employing Bechold's ultrafilter collodion membranes estimated that the size of the units of virus was between 20 and 100$\mu\mu$. Levaditi, Nicolau and Galloway (1926[1]) and Olitsky and Boëz (1927[2]) have reported on filtration through collodion sacs but no very significant results have been obtained by this technique.

The electrical charge carried by the virus.

Olitsky and Boëz (1927[2]) stated that the isoelectric point of the virus is about pH 8. At pH values from 6·6 to about 8 the virus carried a positive charge; at pH 8·11 it was negatively charged. There is no published confirmation of these results; on the contrary Burbury (personal communication) has found that from pH 6·6 to pH 9·4 the virus was negatively charged.

Titration of the virus by inoculating guinea-pigs.

Titration may be easily accomplished by intradermal inoculation of the plantar pads of guinea-pigs. Virus can be rubbed on the surface of the pads after they have been scarified, but a preferable method is to inoculate intradermally by running a blunt fine needle attached to a syringe through the epidermis as superficially as possible along the whole length of the pad, piercing the surface at the far end. The needle track can be left full of virus by injecting as the needle is withdrawn. Three or more tracks may be made on each pad. The amount of virus thus inoculated cannot easily be measured but it is sufficiently constant to allow the potency of the virus to be expressed as the minimal infective dilution. When inoculation is made intramuscularly or by any route other than the intradermal the amount as well as the dilution of the virus should be specified. It is probable, however, that other routes are less satisfactory. For a fuller discussion of titration see Bedson, Maitland and Burbury (1927).

Alterations in the virus due to passage through different species.

Virus taken from cattle, sheep or swine is much less infective for guinea-pigs than the same strain after it has been adapted to guinea-pigs by several passages. Usually after 6 to 10 passages the virus will produce the typical disease in guinea-pigs, and the filtered vesicle fluid from the primary lesions will have a titre of about 1 in 5,000,000 (titrated by intradermal inoculation of the plantar pads). The type of virus, determined by cross-immunity tests, is maintained after adaptation to guinea-pigs.

So far it has not been possible to attenuate virus from cattle by passage through a lower or other species so as to make it employable as an immunizing agent for cattle.

The effect on the virus of various physical factors.

Hydrogen-ion concentration. The optimum reaction for conservation of the virus is pH 7·5 to pH 7·6 (Bedson and Maitland, 1925 ; Stockman and Minett, 1926[1] ; Olitsky and Boëz, 1927[4] ; Schoening, 1927). Even small variations of acidity or alkalinity are rapidly destructive. Virus in a phosphate solution at pH 6·2 survived at 0 to 4° C. for less than 69 hours ; at a reaction of pH 7·6 it survived for 96 days at least (Bedson and Maitland, 1925). The acidity produced by grinding muscle in sand, (M. C. Maitland, 1928), and the acidity of muscle associated with *rigor mortis* (Stockman and Minett, 1927) rapidly destroyed the virus.

Centrifugation. Contrary to the views of Frosch and Dahmen (1924[1 & 2]) the general opinion is that virus is not deposited by centrifugation (Gins, 1924[1] ; Stockman and Minett, 1925, 1926 ; Bedson and Maitland, 1925 ; Olitsky and Boëz, 1927[1]).

Light. Light has a viricidal action. Even winter light (London) transmitted through glass reduced the period of survival of dried virus (Bedson, Maitland and Burbury, 1927). Ultraviolet light (wave-lengths of from 5,720 to 2,300 A.U.) caused a 1 in 10 dilution of filtered vesicle fluid from guinea-pigs to become non-infective in 5 minutes. Virus in serum or in unfiltered vesicle fluid was to some extent protected (Galloway and Nicolau, 1928).

Heat. Burbury (1928) has demonstrated that the destruction of foot-and-mouth virus by heat takes place according to laws which apply to bacteria. At 50° C. the rate of destruction proceeds in an orderly manner, the velocity constant being 0·0108. Under any given conditions the rate of destruction is influenced by the concentration alone, the amount destroyed in a given time being proportional to the amount present. The effect of temperature upon the velocity of destruction is uniform and can be expressed as a temperature coefficient. Between 50 and 60° C. the coefficient was 1·7, i.e. the velocity of destruction was increased 1·7 times for each rise of temperature of 1° C. A coefficient of the same order (1·6) was found by Chick (1910) for *B. typhosus.* The actual times required to kill virus cited by Burbury agree in general with the earlier observations of Loeffler and Frosch (1898) and Matte and Sanz (1921).

Cold. The virus is resistant to cold, and low temperatures are best for storing it. Virus withstood freezing and thawing at least 20 times (Stockman and Minett, 1926[1]), and remained infective at ±5° C. for 433 days (Bedson, Maitland and Burbury, 1927). Lebailly (1921), Stockman and Minett (1926[1]), Roux, Vallée, Carré and Nocard (1921) and Gins and Krause (1924) have made similar observations. The earlier literature has been summarized by Gins and Krause (1924).

Adsorption. Vallée and Carré (1921) reported that virus was readily adsorbed from vesicle fluid by ox red blood corpuscles or by killed staphylococci or pneumococci. Abe (1925) employed kaolin and animal charcoal with similar results. The adsorption of virus by red cells was not confirmed by Bedson and Maitland (1925) or by Stockman and Minett (1926[1]). The latter authors also failed to confirm adsorption by killed staphylococci. Gins and Krause (1924) obtained no adsorption with kaolin, and Frosch and Dahmen (1924[2]) likewise found that red corpuscles, kaolin and animal charcoal did not adsorb the virus. Bedson and Maitland (1925) were not able to detect adsorption either on the precipitate produced by adding guinea-pig antiserum to guinea-pig vesicle fluid or that which formed after the addition to it of alcohol. Stockman and Minett (1926[1]) obtained a loose adsorption of virus on kieselguhr. Bedson, Maitland and Burbury (1927) secured adsorption with a semi-opaque suspension of globulin, precipitated from guinea-pig serum with ammonium sulphate, but if the particles of globulin were dissolved in a sufficient volume of saline the virus was liberated.

Oxygen tension. Bedson and Maitland (1925) concluded that reduction of the atmospheric pressure to 50 or 30 mm. Hg did not materially alter the period of survival of virus in buffer phosphate solution, pH 7·6 at 37° C. Stockman and Minett (1926[1]) were in agreement. Olitsky and Boëz (1927[4]), on the other hand, found that under strict anaerobic conditions the virus retained its infectivity for at least twice as long as under aerobic conditions. The discrepancy in results they attribute to the special anaerobic technique which they employed. The oxygen of the air did not prevent the survival of dried virus for periods of over one year provided the atmosphere was chemically dry (Burbury, 1928).

Drying. Survival of the virus in the dried state is conditioned by the medium in which the virus to be dried is suspended, the temperature and the rate at which it is dried, the temperature and moisture of the atmosphere in which it is kept in the dried state, whether or not it is exposed to light, and notably also by the material on which it is dried. These facts have arisen from the extensive experiments on drying made by Burbury (1928). An atmosphere saturated with moisture caused more rapid death of virus than one 70 per cent. saturated. Replacing the air with hydrogen did not preserve the virus, in the presence of 70 per cent. moisture. In chemically dry air the oxygen did not prevent survival for more than one year. Destruction of virus in the dried state appears, therefore, to be caused by chemical activity in or around the virus occasioned by the presence of moisture. Certain materials, especially hay and bran, favoured the survival of virus dried on them and the periods for which they remained infective were sufficiently long to implicate them as possible conveyers of the disease. The reason for the longer survival of virus on hay, bran, flour, sugars and cow hair than on other substances has not been fully explained, but the suggestion was made that an explanation may be found in the action of dehydrating agents which they are known to contain. Trautwein (1926[1]) found that virus in epithelium from vesicles exposed to atmospheric conditions in the open in winter remained infective for 67 days. Schoening (1927) reported that under field conditions virus remained infective in hay or garden soil for 25 to 30 days. References to earlier observations, which on the whole have been less comprehensive than Burbury's and with which hers are in general agreement, are given in her article.

The action of chemicals. Disinfectants.

In the literature there are details of many experiments made to test the effect of a large variety of chemicals on the virus. As a rule the experiments of recent date in which the virus has been titrated are more valuable than the earlier records, of which a summary has been made by Gins and Krause (1924).

Glycerol. Glycerol preserves the virus in an acid medium in which without glycerol it would rapidly become non-infective (Bedson and Maitland, 1925). At pH 7·6, 50 per cent. glycerol has little if any effect. A higher concentration (96 per cent.) in these circumstances was deleterious (Bedson, Maitland and Burbury, 1927). Stockman and Minett (1926[1]) obtained similar results.

Chloroform and ether. The virus is very resistant to chloroform (Stockman and Minett, 1926[1]; Bedson and Maitland, 1925; Bedson, Maitland and Burbury, 1927) and to ether (Schmid, 1926).

Alcohol. Alcohol is less destructive to the virus than to bacteria (Stockman and Minett, 1926[1]; Bedson and Maitland, 1925; Abe, 1925).

Olitsky and Boëz (1927[3]) are of opinion that the resistance of the virus to alcohol is only apparent, and that if the coagulation of protein be prevented by the addition of sodium hydrate the virus is rapidly destroyed by alcohol. Minett (1928) quotes one experiment, out of several attempted, in support of this view, but it has not been generally confirmed.

Formaldehyde. The virus is easily killed by formaldehyde (Vallée, Carré and Rinjard, 1925; Bedson and Maitland, 1925; Stockman and Minett, 1926[1]; Bedson, Maitland and Burbury, 1927).

Other reagents. Galloway and Nicolau (1928) stated that ox-bile and various aniline dyes were weakly destructive, and that potassium tellurite and sodium selenite were much less destructive to the virus than to bacteria. They, as well as Maitland (1928), have found the viricidal action of sodium ricinoleate to be weak. Bedson and Maitland (1925) found the virus to be more resistant to phenol than most bacteria, in agreement with the observation of Loeffler and Frosch (1898).

Chemicals investigated as disinfectants. Stockman and Minett (1927) and Minett (1928) have reported on the action of about forty chemicals, alone and in the presence of a mixture of saliva and fæces, and have tested the disinfecting power of some of them on dried virus. Olitsky and Boëz (1927[3]) made a special point of the resistance of virus to chemicals which coagulate protein. They considered that such coagula protected the virus. As disinfecting agents they favoured sodium hydroxide or antiformin.

Trautwein (1925, 1926[2], 1928[1 & 2]) has carefully studied numerous disinfectants and has brought forward sulphurous acid and its derivatives as having a powerful and selective action. He considered them to be superior to all the other chemicals he had tested. 'Sulfoliquid D.S.' (a mixture of sodium or potassium bisulphite and bisulphate with lactic acid) was particularly recommended, and also powders consisting of sulphurous acid compounds with metallic salts of an acid reaction. 'Rohchloramin' was less effective. A 1 per cent. solution of sodium hydrate was considered to be a very useful disinfectant and could be sprayed on animals without harming them. Helm and Wedemann (1928) confirmed in general the usefulness of 1 per cent. sodium hydrate and tested cresolsulphonic acid and formaldehyde with favourable results. Their results with 'Sulfoliquid' did not confirm Trautwein, who attributed their different results to technique. The best preparations they tried were 'Sulfalyd' 1 and 2 (a condensation product of formaldehyde and sulphurous acid with or without lactic acid). Weiss (1926) found 'Chloramin' and 'Serapid' to be very active owing to the acidity produced by them.

Cultivation experiments.

Attempts have been made by many to cultivate the virus, but without success. The earlier literature has been reviewed by Gins and Krause (1924). In 1924 the claim of Frosch and Dahmen received attention and with it the earlier work along similar lines of Guth (1924), Titze (1922, 1924) and Pfeiler (1922). Titze considered that multiplication of the virus had been demonstrated through complement-fixation tests although his cultures failed to infect animals. Guth found that if inoculation of the culture failed to produce lesions, passage of the inoculated tissue, in series, in animals might result in their appearance. Pfeiler considered that he had succeeded in infecting one animal with a culture that had been at room temperature for 200 days. These claims have not been confirmed and there is not, on the whole, sufficient evidence in them that the virus had undoubtedly been propagated *in vitro.*

Frosch and Dahmen (1924[2]) used a serum-agar medium and obtained minute transplantable colonies. With the seventh subculture they claimed to have infected animals ; and once, with the twenty-third subculture, by passage of the inoculated tissue in series in guinea-pigs, vesicles developed. Ultraviolet light was used to photograph the units of virus in the cultures. Their work was not confirmed, however, by a German Commission (Gins, 1924[1], 1925), nor were Bedson and Maitland (1925) and Stockman and Minett (1925, 1926[1]) able to repeat their findings. The colonies seen by Frosch and Dahmen were probably the artefact colonies described by Laidlaw (1925).

Further unsuccessful attempts to cultivate the virus have been made by Bedson and Maitland (1927) and by Olitsky and Boëz (1927[4]). Burbury (1928) was unsuccessful in obtaining growth of the virus in cultures containing cysteine, introduced with the object of regulating the oxidation-reduction potential.

Immunity, Natural and Acquired. Specific Prophylaxis and Serum Therapy.

Immunity in cattle, sheep and pigs.

It is probable that cattle, sheep and pigs have very little natural immunity against foot-and-mouth disease, but individuals vary in degree of susceptibility. Cattle which have recovered from infection are believed to be immune for from one to two years. The duration of immunity in other naturally susceptible species has not been determined. Animals which have recovered from disease caused by one type of virus are immune against that type only, although a small degree of cross-immunity for the other types may occur (Trautwein, 1927).

Experimental immunity in the guinea-pig.

The grades of immunity in guinea-pigs. The nature of immunity in the guinea-pig appears to be the same as in cattle. Its degree can be divided quantitatively into three broad grades, according to the size of the test dose of virus and the route by which it is given. The highest grade is represented by complete resistance to intradermal inoculation of the plantar pads and can be stated precisely as complete resistance to a certain number of minimal infective doses. A lower grade is indicated by absence of secondary lesions following a primary lesion on the plantar pads. This, it has been suggested, corresponds most nearly to that degree of immunity which in cattle would be sufficient to protect them against natural infection (Bedson, Maitland and Burbury, 1927). The lowest grade is demonstrated by the absence of lesions following an intramuscular inoculation of an amount of virus just sufficient to infect normal guinea-pigs by this route.

Immunity after recovery. After 8 to 9 months the majority of guinea-pigs (5 out of 6) were completely resistant to 500 minimal infective doses of virus given intradermally, and of 13 tested all had immunity of the second grade. Further details are to be found in the original paper (Bedson, Maitland and Burbury, 1927).

Passive immunity. From 0·2 to 0·5 c.cm. of serum from recovered guinea-pigs given intramuscularly, subcutaneously, intravenously, or intraperitoneally to normal guinea-pigs 18 to 24 hours before inoculation of their pads with virus will protect the majority against generalized lesions. It is not possible completely to protect guinea-pigs passively against the intradermal inoculation of more than 2 to 5 minimal infective doses of virus, even with large doses of serum (Bedson, Maitland and Burbury, 1927).

Active immunity produced by vaccine (killed virus) with and without subsequent inoculations of living virus.

The preparation of vaccine. Vallée, Carré and Rinjard (1925, 1926[1 & 2]) protected calves against infection by inoculating them with a formolized emulsion of tongue epithelium taken from an infected calf. At this time Bedson and Maitland (1927) had begun independently to investigate the value of virus killed by formalin as a prophylactic vaccine for guinea-pigs. The potency of formolized vaccine will depend primarily on the quantity of virus in the material used for its preparation. The virus may be killed by exposure to 0·1 per cent. formalin (40 per cent. formaldehyde) at 26° C. for 48 hours. Large doses then fail to infect guinea-pigs. An exposure longer than the minimum required to kill the virus tends to destroy the efficiency of the vaccine. The best results have been obtained when the reaction of the suspending medium was pH 7·6. Virus killed without formalin by exposure to a reaction of pH 6·5 was useless as vaccine. The efficiency of a formolized vaccine was destroyed by altering the reaction to pH 6·5 and keeping it at 37° C. for 24 hours. This harmful effect of acidity is not reversible. Virus suspended in a phosphate buffer solution at pH 7·6 and kept at 37° C. until it was dead was about equal in potency to formolized virus. Virus killed with 2 per cent. phenol at 26° C. and at 37° C. pH 7·4 conferred very little immunity. Drying the virus either at 37° C. or at 18 to 20° C. very greatly reduced its immunizing properties. Virus which had died after several days' incubation in an experimental culture medium at 37° C. and virus killed by heating at 55° C. for 45 minutes were very weak immunizing agents. The above facts were demonstrated by Bedson, Maitland and Burbury (1927) and Maitland (1928).

The preparation of vaccine with sodium ricinoleate was tested on account of the work of Larson and others (1926) and Netter, André, Césari and Cotoni (1927) on the lethal effect of ricinoleate on bacteria. The virus is more resistant to this reagent than bacteria are said to be and virus killed by it is probably less efficacious as vaccine than formolized virus (Maitland, 1928).

Testing the potency of vaccine. The potency of samples of vaccine can be estimated by determining the proportion of guinea-pigs which a certain dose will protect. The vaccine is given intramuscularly, and one week later a strong virus is inoculated intradermally on the plantar pads. Protection is indicated by the absence of generalized lesions. The following results were obtained with an average sample of vaccine. Filtered vesicle fluid from guinea-pigs in phosphate solution, pH 7·6, with a titre of 1 in 1,000,000 was killed by exposing a 1 in 50 dilution to 0·1 per cent. formalin at 26° C. for 48 hours; 0·01 c.cm. protected none of 16 guinea-pigs, 0·1 c.cm. protected 15 of 29, 0·5 c.cm. protected 15 of 18, and 1·0 c.cm. protected each of two (Maitland, 1928).

The storage of formolized vaccine. Maitland (1928) tested two vaccines after storage for seven months. At room temperature each had much deteriorated; at ± 5° C. there was very little if any deterioration.

Immunity following the inoculation of formolized vaccine. Definite increase in resistance of guinea-pigs has been detected two days after vaccination (Bedson, Maitland and Burbury, 1927). The immunity produced is specific for the immunological type of virus from which the vaccine is prepared. The degree of immunity which follows one inoculation of vaccine is relative to the size of the dose. After several inoculations complete resistance to intradermal inoculation of 10 and 100 minimal infective doses has been recorded. The duration of immunity is conditioned by the size and number of inoculations given (Bedson, Maitland and Burbury, 1927).

Immunity following the inoculation of subinfective doses of living virus alone or preceded by formolized vaccine. Subinfective doses of living virus are more effective in producing immunity in guinea-pigs than corresponding amounts of virus killed by formalin. The infectivity of virus is reduced by mixing it with immune serum, but such mixtures are less efficacious than corresponding amounts of living virus (Bedson, Maitland and Burbury, 1927). By giving repeated inoculations of living virus after one or more initial inoculations of formolized vaccine Bedson, Maitland and Burbury (1927) showed that a degree of immunity equal to that produced by an attack of the disease can be built up without the occurrence of lesions.

Adjuvants to vaccine. The experiments recorded by Maitland (1928) suggest that formolized normal serum, casein or plague vaccine may each on occasion act as adjuvants if inoculated at the same time as formolized vaccine.

Demonstration of antibodies against the virus.

Ciuca (1929) has shown that the serum of recovered or hyperimmune guinea-pigs will fix complement specifically *in vitro*. Antibodies can also be demonstrated by passive immunization and by the viricidal action of immune serum *in vitro*. The last two methods can be made to serve for estimating the strength of antibodies. Waldmann and Trautwein (1928[2]) have reviewed the literature of the methods of testing the strength of serum and describe a method based on passive protection of guinea-pigs used at the Foot-and-Mouth Disease Station in the Island of Riems. The route by which the serum is inoculated, the length of time between the inoculation of serum and the test dose of virus, the size of the test dose, and the route of giving it, should be kept constant, and a sufficient number of guinea-pigs should be used for each determination to compensate for individual differences. An alternative method is to mix serum with a series of falling dilutions of virus. After they have stood in contact at room temperature for 20 to 30 minutes each dilution is inoculated intradermally into the plantar pads of a guinea-pig. By determining the reduction of titre due to admixture with serum it is possible to express the neutralizing power of serum as the equivalent of a certain number of minimal infective doses of virus. This method is described in detail by Maitland and Burbury (1927) and has been used to investigate the relation of antibodies to the various states of immunity.

The relation of antibodies to immunity.

The relative importance of antibodies, leucocytes and fixed tissue cells in determining immunity to foot-and-mouth disease has not been fully investigated, but humoral immunity is probably the most important factor. An amount of antibody which will protect passively when introduced into the circulation of a guinea-pig, may be in too low a concentration in the protected animal for detection in the blood (Maitland and Burbury, 1928). Whether the immune serum neutralizes the virus in the animal by combining with it directly or through the agency of leucocytes or fixed tissue cells which have been influenced by it has not been determined.

The serum of guinea-pigs tested *in vitro* one week after receiving 0·5 c.cm. of formolized vaccine cannot be shown to contain any more antibodies than are present in normal serum. These animals, however, do not develop generalized lesions after intradermal inoculation, and when they are inoculated intravenously no virus is detectable in their blood after 30 minutes. The mechanism of this immunity and the rapid removal of virus has not been explained, although it is probable that antibodies are present in amounts too small to be detected in the shed blood. Antibodies have been demonstrated in the blood of guinea-pigs after they have received several inoculations of vaccine (Maitland and Burbury, 1928). Whole blood from recovered animals is slightly more destructive to virus *in vitro* than the corresponding serum and it is possible that leucocytes may aid in its destruction. In the rapid removal of virus from the circulation reticulo-endothelial cells may be concerned (Maitland and Burbury, 1928).

After recovery from an attack antibodies persist in the serum for several months (Maitland and Burbury, 1927).

The serum of guinea-pigs which had been immunized by one dose of formolized vaccine followed by several subinfective doses of living virus neutralized *in vitro* as much virus as the serum of recovered animals. A few experiments have been made which indicate that the globulin content of immune serum is increased (Maitland and Burbury, 1927).

Plurality of types of virus.

Vallée and Carré (1922[1 & 2]) indicated the existence of two types of virus, which could be distinguished by the failure of a state of immunity towards one to protect against the other. The infections could not be distinguished clinically with any certainty although minor differences were suggested. The types were designated ' A ' (allemand) and ' O ' (Oise). These original observations have been amply confirmed and extended. Waldmann and Trautwein (1926) described three types of virus obtained from outbreaks in Germany and labelled them ' A ', ' B ' and ' C '. Trautwein (1927) investigated 76 strains obtained from several countries and found that they were all included in the three types. The differences in type were sharply defined when tested by cross-immunity experiments in guinea-pigs, but were sometimes less sharply marked when tested in swine, and might be still less so in cattle, but in no case was a reference to one of the types doubtful.

The relation of the types ' A ' and ' O ' to ' A ', ' B ' and ' C ' has been investigated by Maitland, Burbury and Bedson (1927), Burbury (1928) and Trautwein (1927). Vallée and Carré's types ' A ' and ' O ' correspond to Waldmann and Trautwein's types ' B ' and ' A ' respectively. Thus three types exist, and Vallée and Carré (1928) state that it has been agreed, to save confusion in nomenclature, to designate them ' A ' and ' O ' (Vallée and Carré) and ' C ' (Waldmann).

Geographically the types are distributed irregularly.

Methods of determining the type to which a strain of virus belongs. The basis of the methods by which the type of a strain of virus may be determined is cross-immunity. The experiments may be made on any susceptible species (cattle, sheep, swine and guinea-pigs have been used), and animals may be immunized by infection, by vaccine, or passively, with immune serum. Complement fixation has also been successfully employed (Ciuca, 1929) for this purpose.

There is no indication that a strain of virus alters in type by passage through another susceptible species, but clear-cut differentiation of types can only be expected if attention is paid to the virulence of the strains examined and the degree of immunity of the animals employed. Apparent variation from type may be quantitative rather than qualitative. When species which are naturally susceptible are used the complication of unrecognized previous mild disease producing partial immunity may interfere. Trautwein (1927) attributed apparent variation of some strains to this cause when he employed cattle and swine. The results of typing strains on guinea-pigs have been shown to apply to cattle and other animals (Trautwein, 1927; Stockman and Minett, 1926[2] and 1927). A fuller discussion of typing strains by employing guinea-pigs will be found in the articles of Maitland, Burbury and Bedson (1927), Burbury (1928) and Trautwein (1927).

Passive immunity in cattle, sheep and pigs.

Serum from recovered or hyperimmune cattle will protect against natural infection for about two weeks. The German serum is now polyvalent and is obtained by bleeding-out cattle which have recovered from an infection produced with known strains. It is not concentrated. Its potency is tested on guinea-pigs as described in an earlier section. The dose recommended by Waldmann (1925) is 20 c.cm. per hundredweight; amounts greater than this are said not to be more advantageous. Many favourable reports on the use of serum have appeared in the German literature.

Active immunity in cattle, sheep and pigs.

The inoculation of a virus of low virulence, in order to produce a mild attack of the disease, has been practised occasionally, but the method cannot be considered safe as a general procedure. A method recommended by Waldmann (1926) for the control of outbreaks in Germany is termed 'simultane Heil- und Schutzimpfung'. The results of experimental investigation of this method have been recorded by Reppin (1927). The whole scheme, in outline, is to inspect all the animals at the site of an outbreak. Those with lesions may be given serum. Those which have fever are inoculated with serum. Those without lesions or fever are inoculated on the lip with virus from one of the infected animals and at the same time are given antiserum. Animals on farms surrounding the infected area should also be inoculated with serum in order to prevent the spread of the disease.

The protection of farm animals by the inoculation of formolized vaccine is still in the experimental stage, although Vallée, Carré and Rinjard (1926[1 & 2], 1928) and Stockman and Minett (1927) have indicated that cattle can be protected by vaccine. The primary difficulty in applying vaccination in the field is that at present vaccine can be prepared only from the tissues or serum of infected animals. Vallée, Carré and Rinjard (1928), however, using the carcasses of guinea-pigs, have made 500 c.cm. of vaccine from one animal.

Vallée and Carré (1928) have found that some cattle, after recovery, possess a hypersensitiveness of the epithelial tissues, and they have also drawn attention to a state of general hypersusceptibility produced by repeated inoculations of living virus at short intervals, in cattle which have recovered from infection. This is difficult to explain and requires further careful experimental consideration.

Serum therapy.

The treatment of the disease with hyperimmune serum or serum from convalescent animals is of limited value. Given early in the disease it may decrease the severity of the lesions and the general reaction.

Chemotherapy.

No specific chemotherapeutic substance has so far been found for foot-and-mouth disease, although considerable work in this direction has been done. There are no clues as to the kind of compound, if any, which might be more likely than another to act against this virus *in vivo.*

Waldmann (1928) investigated about sixty compounds without finding any that influenced infection. Jacob (1926) and Steinhoff (1927) reported favourably on the use of Parenchymatol M.K. Gins (1924[2]) tested bismuth preparations in guinea-pigs, and stated that Heyden 531, 564B and 590 influenced favourably the severity of infection, and that Heyden 590 might prevent infection if given before the guinea-pigs were inoculated with virus. He did not test the compounds in cattle. Saxinger (1924) confirmed Gins's findings, but found the three bismuth preparations inactive against the virus *in vitro.* He suggested that their beneficial action was due to leucocytosis caused by their injection and believed that they had no direct effect on the virus *in vivo.* Kraus (1926) found a bismuth preparation obtained from Merck and labelled 'X' to be without beneficial effect. Walker and Taylor (1927) claimed that the intravenous injection of an aqueous solution of iodine and potassium iodide in the febrile pre-eruptive stage of the disease would in calves prevent the development of lesions and cut short the infection. Confirmation could, however, not be obtained in guinea-pigs (Maitland, 1928) nor in cattle in this country.

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CHAPTER VI. VESICULAR STOMATITIS OF HORSES.

BY H. B. MAITLAND (UNIVERSITY OF MANCHESTER).

Clinical Description.

Horses and mules. In the past there has undoubtedly been confusion in the nomenclature of stomatitis of horses. The disease described here is distinct from that investigated by de Jong (Stomatitis pustulosa contagiosa equi) and identified by him and also by van Heelsbergen (1922–3) with vaccinia in cattle. The recognition that vesicular stomatitis was caused by a filterable virus (Cotton, 1926[2]; Olitsky, Traum and Schoening, 1926) has enabled its characteristics to be delimited.

Mohler (1918) described the natural infection in horses. Olitsky, Traum and Schoening (1926) and Cotton (1926[2]) have studied the course of the disease after inoculation of the tongue, and Cotton (1926[1]) demonstrated virus in the blood of experimentally infected horses. The disease in horses conforms clinically to foot-and-mouth disease in cattle.

Cattle. Vesicular stomatitis is sometimes transmitted naturally to cattle from the horse and they can regularly be infected experimentally. Articles by Olitsky, Traum and Schoening (1926), Mohler (1918) and Cotton (1926[1]) describe the clinical features, which cannot be differentiated with certainty from foot-and-mouth disease.

Swine. Olitsky, Traum and Schoening (1926) confirmed the earlier observations of Mohler (1918) that swine were susceptible and that the disease was clinically indistinguishable from foot-and-mouth disease.

Guinea-pigs. Guinea-pigs are susceptible and can be infected in series indefinitely (Cotton, 1926[1]; Olitsky, Traum and Schoening, 1926; Olitsky, 1927). There is no certain clinical distinction between vesicular stomatitis and foot-and-mouth disease in the guinea-pig, but secondary lesions occur less commonly with vesicular stomatitis.

Rabbits, rats and mice. Cotton (1926[1]) was not successful in infecting rabbits or white rats and mice. Olitsky (1927), however, infected rabbits in series. The cornea was susceptible, but the virus could be differentiated from herpes virus.

Epizootiology.

Cotton (1926[1]) and Olitsky, Traum and Schoening (1926) observed contact infection in cattle. Their experiments suggested that, like foot-and-mouth disease, vesicular stomatitis was communicable before, and for a short time after, the appearance of the lesions, but not in the later stages. Cotton (1926[1]) and Mohler (1918) appear to have demonstrated that stables which have recently harboured infected animals are not sources of infection. The observations of Mohler (1918) suggest that vesicular stomatitis is less contagious than foot-and-mouth disease.

Pathology.

The nature of the epithelial lesions of vesicular stomatitis in cattle, swine and guinea-pigs are identical with those of foot-and-mouth disease (Chapter V, p. 65) (Olitsky, 1927; Olitsky and Long, 1928[1]). Olitsky (1927) noted the liability to secondary infection, a matter to which reference has been made in the chapter on foot-and-mouth disease.

The Nature and Properties of the Virus.

The virus of vesicular stomatitis, as it is affected by physical and clinical agents, behaves similarly to the virus of foot-and-mouth disease. Filtration has been studied by Cotton (1926[2]), Olitsky, Traum and Schoening (1926) and Olitsky (1927). The electrical charge carried by the virus, the effect of hydrogen-ion concentration, centrifugation, and titration have been investigated by Olitsky (1927). The action of glycerol, alcohol, sodium hydroxide and other chemicals on the virus and its survival outside the body were also considered by Olitsky (1927). The effect of ultraviolet light has been studied by Olitsky (1927) and Olitsky and Gates (1927). The virus can be passed indefinitely through susceptible species (Cotton, 1926[1]).

Carrel, Olitsky and Long (1928) obtained evidence of growth of the virus in tissue cultures of guinea-pig embryo tissues or bone-marrow from adult guinea-pigs.

Immunity.

Cotton (1926[1]) found two horses to be solidly immune 1 year and 5 months respectively, after recovery, and immunity to reinfection was demonstrated in cattle 10½ months after recovery. Olitsky (1927) stated that guinea-pigs were completely immune 4½ months after an attack of the disease.

Olitsky, Traum and Schoening (1926) showed that immune guinea-pig serum neutralized virus *in vitro* and that 0·1 c.cm. of this serum would protect guinea-pigs passively to the extent of preventing generalized lesions.

Subinfective doses of living virus readily induce a degree of active immunity sufficient to prevent infection. This has been demonstrated for cattle (Olitsky, Traum and Schoening, 1927); for horses (Cotton, 1926[2]) and for guinea-pigs (Long and Olitsky, 1928[1]).

Active immunity produced by the inoculation of formolized virus has been studied by Olitsky and Long (1928[2]). The virus of vesicular stomatitis was more resistant than that of foot-and-mouth disease to formalin, and they concluded that dead virus failed to produce immunity in guinea-pigs even after repeated inoculations. Their criterion of immunity was the absence of primary lesions. In criticism it should be said that dead formolized foot-and-mouth virus, in similar amounts does not prevent primary lesions if more than 2 to 5 minimal infective doses are given, but that it does prevent generalized lesions. Their conclusions appear, therefore, to require reconsideration.

Olitsky, Traum and Schoening (1926) have demonstrated that no cross-immunity exists between vesicular stomatitis and foot-and-mouth disease. Types ' A ' and ' O ' of the latter virus were tested, but the relation of type 'C' (see Chapter V, p. 76) to vesicular stomatitis has not been investigated.

Differential Diagnosis.

Foot-and-mouth virus which has been adapted to the guinea-pig usually produces generalized lesions, but the virus of vesicular stomatitis frequently fails to do so. This is probably not an absolute criterion. Some strains of foot-and-mouth virus produce fewer generalized lesions than others and not many strains of vesicular stomatitis virus have been tested. Foot-and-mouth virus is not, or only with great difficulty, transmissible to the horse.

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CHAPTER VII. SMALLPOX.

(Variola; Petite Vérole; Blattern.)

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Symptomatology.

Description. Smallpox is an acute infectious disease characterized by fever and a general eruption which in some eight days passes through the stage of papule, vesicle and pustule, affecting the skin and mucous membranes.

Course and symptoms. The disease in its typical form runs a well-defined course which may be divided into four stages:

1. The incubation stage, which is as a rule 12 days, varying from 10 to 13, reckoned from the date of exposure to the date of onset.

2. The initial stage of toxæmia, which lasts from 4 to 6 days, acute during the first 2 or 3, then gradually subsiding. The onset may be gradual or abrupt, with headache, giddiness, rising temperature, backache, and gastric disturbance. During this stage rashes may occur. These are of a toxic nature and are of two kinds: (*a*) purpuric or petechial; (*b*) erythematous.

3. The stage of true exanthem or focal eruption. This is first apparent on the third or fourth day of the illness. The distribution of the rash is liable to many variations, but it generally appears first on the face and hands, extends downwards and usually is not found on the legs till 24 hours later. The first recognizable sign is a spot of the size of a pin's head, not raised and not to be felt. Enlargement rapidly follows and the spot becomes first a papule, raised, hard to the touch, and reddish in colour, then vesicular containing a clear fluid; it is often loculated and umbilicated, but not necessarily so. It still grows larger, and after some 24 hours the contents become turbid and by the sixth day appear purulent, so that each vesicle becomes what is called a pustule. Concurrently with that on the skin an important manifestation of the focal eruption may appear on the mucous membranes of nose, mouth, fauces, pharynx, larynx, trachea, bronchia and genitalia, also sometimes on the conjunctivæ.

4. The stage of secondary fever or suppuration, which manifests itself on the sixth or seventh day of the efflorescence, when the temperature begins to rise again. From the eighth to the ninth day the lesions show signs of drying up and leave scabs which eventually separate and fall off about the fourteenth day. It is customary to speak of a vesicle when its clear contents become turbid as being transformed into a pustule

and the process as pustulation, which suggests that the contents of the mature pock are puriform. This does not seem to be the case. Polymorphonuclears form but a minor proportion of the contents, indeed, they are often conspicuous by their paucity, and the turbidity is mostly due to invasion of small mononuclears of hyaline type. Pyogenic cocci are frequently not to be found in the lesions, and, even when present, polymorphonuclears are seldom abundant. The suppurative fever, when it occurs, seems to arise not so much from the skin lesions as from the lesions of the mucous membranes of the respiratory tract, where denudation of the surface is often extensive, and pyogenic organisms, especially the streptococcus, may be abundant.

Infection takes place in nearly all instances through the respiratory tract. Cases have been noted which followed direct ingestion of infected material or direct contact with the infected skin, as from a handshake, but such occurrences are quite exceptional.

The infective particles, air borne, impinge on the nasal or buccal mucosæ, or may be conveyed thither by the hand after contact with infective material. At the point or points of impact no local lesion arises and this constitutes a fundamental difference between the naturally acquired disease and that artificially inoculated.

In the latter the virus is applied to traumatized epithelium and a local lesion results. In the natural disease the virus apparently passes between the epithelial cells without affecting them, but where it locates itself is unknown. Reproductive growth must take place on a pronounced scale, but is unaccompanied by symptoms, and maturity is not reached till the twelfth day, when onset of illness begins. This is due to discharge of toxin derived perhaps from sporoblasts brought about by sexual conjugation of the virus. The toxin gives rise to the constitutional symptoms and fever, and has a vasomotor and angiolytic action evidenced by initial petechial rashes and by the hæmorrhagic forms of smallpox.

Entry of the virus into the blood-stream must follow closely on the toxin invasion, but is not synchronous with it, as shown by the exemption from the focal eruption of areas affected with petechial rashes, due to the way being already blocked by stases in the capillary circulation (Ricketts, 1908). The virus is precipitated in the capillary circulation, especially, it may be assumed, where the endothelial lining of the vesicles has been previously injured by the toxin. The precipitation is perhaps partly embolic, as suggested by the clustering of the focal eruption at the edges of irritated patches, and the preference shown for the more vascular parts consequent on exposure to air and sunlight, such as face and hands. It is not easy, however, to correlate the somewhat curious distribution of the exanthem in smallpox with a purely mechanical circulation of the virus. On the lower limbs, for instance, the exanthem may not appear till 24 hours after that on the face. No adequate explanation of this delay has been advanced. Possibly deficiency in oxygenation may have some influence, or differences in texture of skin. The regular yet rapid development of the lesion suggests that it is due to a multiplication of the virus by schizogony, and no doubt a toxin is secreted which probably has the power to antagonize phagocytosis, thereby facilitating the growth of pyogenic organisms. That some action of this nature takes place is seen in the course of smallpox in vaccinated patients, in whom the disease instead of developing fully is modified and assumes the form known as varioloid. In such cases, according to the amount of residual immunity, the toxæmia may develop with perhaps little mitigation, but the efflorescence may be almost entirely absent; when there is still less immunity, the toxæmia and efflorescence develop perhaps to the full, but the suppurative process is prevented, and the pocks heal without suppuration. The residual antibody is sufficient to counteract the secondary toxin, though impotent against virus proliferation and vesicle formation. Seldom in the vaccinated does the suppurative fever assume serious proportions.

From about the fourteenth day and onwards the pocks dry up and separate, containing in their desiccated scabs the virus alive and fully able to transmit the disease to a new host, and this is a feature even of those scabs, such as the seeds in the feet and hands, which may be still adherent when the patient has otherwise completely recovered from the disease.

Scabs, however, are not the only source of infection; for from the very onset and throughout the disease the virus seems able to escape from the body endowed with full infective powers, and, as far as can be judged, of equal virulence at all stages.

To elucidate the life-story of the virus throughout the disease further research is necessary. Nearly all previous investigations have been directed towards the production of the traumatized disease, but what seems more to be required are systematic attempts to reproduce the disease in the natural way. The nearest approach to this is probably the Chinese method of insufflation of powdered variolous crusts. Gordon (1925) adapted this method in implanting vaccine on to the uninjured nasal mucosa of rabbits. He found that there was no reaction until about the seventh day, when a nasal catarrh appeared. The nasal secretion contained active vaccine virus plentifully, but abated in 2 or 3 days. Cutaneous immunity was complete when inoculation was made on the tenth day. If variola was experimented with in the same way and the mucosa tested from time to time by repeated passage to other mucosæ, allowing a fair time between each passage, it seems possible that a general disease might be engendered. Although most animals, with the exception of the monkey, appear to be generally refractory to primary inoculation with variola, full development may be achieved by the repeated passage method. The same method applied to inoculation by natural ways might lead to helpful results.

Epidemiology.

In pre-vaccination days in this country, epidemics of smallpox occurred every few years. They varied considerably in virulence. Some were very mild; Jenner (1798) speaks of a 'species of smallpox' that was of so mild a nature that the mortality was practically nil; on the other hand, epidemics occurred with a mortality of from 40 to 50 per cent. Similar conditions were experienced in other countries, but vaccination has greatly altered the occurrence and incidence of smallpox. Epidemics of a periodical nature have ceased to occur, mainly due, no doubt, to vaccination and revaccination and to the vigilance of modern sanitary authorities. In this connection it is noteworthy that during and following the War, when in several countries vaccination precautions were necessarily relaxed, smallpox rapidly exhibited prevalence, as in Russia, Italy and Roumania.

Age. Variola attacks all ages and probably susceptibility is equal at all ages. In pre-vaccination times children suffered more than adults because a large proportion of the adults had already passed through the disease. At the present time in those countries where vaccination in childhood is customarily practised, adults are those most frequently attacked, owing to the vaccinal immunity gained in childhood having to a large extent lapsed, whilst children more recently vaccinated escape.

Sex. There is no marked differentiation in the liability of the sexes.

Climate, race and season. Variola occurs all over the world, and climate appears to exert no marked influence, though prevalence is said to be greater in tropical than in temperate climates. This, however, may be partly due to more lax methods of control. White and coloured races are both susceptible. It has been thought that coloured races, especially negroes, show a greater susceptibility, but here again ignorance and lack of control may play a not unimportant part. It is certain, however, that coloured races have been very severely stricken when smallpox was first introduced amongst them.

In this country variola is generally more prevalent in the cooler months of the year than in the summer, especially at seasons in which cold dry winds are common.

Rogers (1925) finds in India close relationship between a low absolute humidity which is consequent on a low monsoon annual rainfall and subsequent epidemic prevalence of smallpox and vice versa. Dryness of the air, therefore, seems to be a factor in the spread of the disease.

Dissemination of the virus. Variola is highly infectious. In the natural disease infection may be communicated to others: (1) by contact with the patient; (2) by contact with articles handled by, or in the immediate vicinity of, the patient; (3) by contact with healthy people who have been in attendance on the patient—hand, clothing or hair; (4) by contact with dead bodies of smallpox patients; (5) by air-borne infection, (*a*) in proximity to the patient in the same apartment or ward, (*b*) remotely from infective particles carried by the wind.

The disease is infectious throughout its course, perhaps even in the later days of the incubation period, certainly in the initial stage and during the early development of the focal eruption, and very obviously in the incrustation or desquamatory stage. The source from which the infection is derived, however, differs in the various stages. That most commonly recognized is the separating scabs and pustules and the concomitant desquamation of the skin in the final stage. There is abundant experimental proof that the infective virus is contained in this material and that it may remain potentially active for years. Earlier in the disease the origin of the infection is not so obvious. Infection during the incubation period is not infrequently suspected but definite proof is difficult to establish.

In the initial stage and in the early stages of the focal eruption infection is almost certainly derived from the respiratory tract. The expired air is contaminated with nasal, salivary, and bronchial secretions, or these are more forcibly expelled by spitting, speaking, &c. The occurrence of the eruption on the mucous membranes of nose, mouth, &c., in a form and position in which it is readily abraded substantially increases the outflow of the virus.

Another probable means of escape is by transudation through the skin, the organism reaching the surface via the sweat glands or hair follicles, whence it is conveyed to clothes or other objects of contact, or on drying is eventually dispersed in air. It is known that the handling of corpses dead before the specific exanthem has appeared, as in virulent hæmorrhagic forms, is a frequent source of infection, indeed, such cases whether alive or dead are considered by many authorities to be more highly infectious in proportion to the duration of the disease than those that undergo the full course. It seems probable, therefore, that skin transudation may play an important part in the propagation of infection.

That the virus of smallpox may be disseminated in these latter ways lacks at present experimental proof. It may be recalled that in the days of variolation, the body secretions and also fæcal matter were experimented with for the purpose of inoculation with a view to obtain a weakened virus, but always without success. The secretions from the air passages almost certainly contain the virus in not inconsiderable quantity, and it should be a relatively simple matter to settle this point and to determine the quantitative infectivity at different stages of the disease.

Air distribution. It has been shown by Power (1882), Barry (1889), Evans (1893) and others, that smallpox infection may be air borne for considerable distances, and that the direction is influenced by the prevailing wind. It is known also that the virus will withstand drying for long periods.

Morbid Anatomy and Histology.

The gross changes that are found *post mortem* depend upon the stage of the disease at which death has supervened. If death occurs in the initial stage from toxæmia, as in purpura variolosa, the chief lesions found are hæmorrhages and extravasations of blood. As already pointed out the toxin has a pronounced tendency to cause angiolysis. This angiolytic action is not confined to the peripheral circulation of the skin, where it gives rise to the toxic rashes, but may occur in almost every part of the body, causing hæmorrhages often extensive in many of the tissues, though the larger organs—heart, liver, brain, spleen and kidney substance—are, as a rule, exempt.

On the other hand, when death follows after the true eruption and the suppurative process the conditions are very different. The blood is fluid with but little signs of coagulation, the heart and large organs soft and degenerate and showing all the signs of a septicæmia to which these changes are due rather than to the specific disease. In the mucous membranes of the air passages, in the upper part of the œsophagus, and in the rectum, pustules, and ulcers resulting from them, may be present, and in severe cases there may be extensive denudation of the surface.

In the liver and other organs circumscribed foci have been described by Weigert (1874) and others, which are considered analogous to the skin lesions. In the bone-marrow similar foci are said to occur in a large number of cases—86 per cent. of those investigated (Chiari, 1893). In the testes also a cellular infiltration occurs in minute disseminated foci, which is said by Chiari to be quite frequent. Councilman found similar lesions and considered them to have a definite relation with the skin focal eruption, but states that the relation is not so absolute as Chiari thought.

Histology of the exanthematous lesions. These lesions are found in the stratified epithelium of the skin, and the mucous membranes lining the respiratory tract, œsophagus, rectum and genitalia; occasionally the cornea is involved.

Weigert (1874) after careful study considered that the lesion started as a necrotic or diphtheroid degeneration of epithelial cells in the rete Malpighii due to the action of the specific virus or its toxin rather than to inflammation. Unna (1894) and Renaut (1881), on the other hand, regard pock formation as due to an acute inflammatory process. Unna describes a reticulating degeneration of epithelial cells, especially of the prickle cells, in which small vacuoles appear near the nucleus; these increase in size forming a net linking the nucleus to the cell-wall. There follows a second degeneration which Unna called ballooning colliquation, affecting chiefly the cells in the lower part of the rete Malpighii. Ferguson (1904) found the earliest visible lesion to be irregular minute dilatations of lymphatic spaces immediately below and sometimes extending between the deepest cells of the Malpighian layer. He noted that in severe cases the corium from an early period is infiltrated with blood corpuscles as well as leucocytes, and laid stress on the leucocytosis apparent in the skin lesions as well as in the blood. Councilman (1904) and his colleagues carried out a comprehensive study of 54 cases, and considered that the lesion began with degeneration of the cells of the lower layer of epidermis, the earliest form of degeneration taking place in the nucleus. In the skin of cases of purpura variolosa, although no macroscopic lesions were apparent, save in two cases ' a few small beginning vesicles ', similar cellular degenerations were found permeating large areas.

The lesions in mucous membranes were formed in the same way, but the degenerated epithelial cells were rapidly cast off and vesicles were rarely seen, pustules never. Breynaert (1881), however, described pustules down to bronchi of the third degree and Joffroy (1880) found pustules in the bronchi.

Councilman and his colleagues made a careful study of the cytological conditions of the various lesions both in the skin and internal organs. They found constant and well-marked proliferation within the hæmatopoietic organs, which gave rise in the spleen, the lymph nodes and the bone-marrow, to the formation of mononuclear basophilic cells, and in the lymph nodes and the marrow to phagocytic endothelial cells. They state that although this process is present to some degree in other infectious diseases, in variola it is so prominent as to be well-nigh characteristic.

Copeman and Mann (1899) made a histological study of the lesions in a vaccinated calf. In regard to the initial stage of the attack, Mann points out that the lesion appears to involve the tissues from below upwards, the blood-vessels of the dermis being engorged and blocked with leucocytes ; the first change to be noticed is a necrosis of the basal membrane 48 hours after inoculation, and it is mentioned as remarkable that this change occurs beneath the vaccinated area, and at a time when epithelial lesions are yet slight. Years previously Cornil (1879) had pointed out that changes occurred in the corium, consisting in swelling of the endothelial lining of the capillaries, and Renaut (1881) also described dilatation of the capillaries in the corium with œdematous infiltration.

These tentative observations have found endorsement in the more modern view of the histology of the variolous and vaccinal skin lesions that the initial attack is on the reticulo-endothelium and not as was formerly supposed on epiblastic tissue.

Ledingham (1924) in a comprehensive study of the vaccinal lesion as produced by cutaneous, subcutaneous, intracutaneous, intravenous, intraperitoneal and intrasplenic inoculation comes to the conclusion that the reticulo-endothelial system is primarily and dominantly involved, and that this involvement may or may not be accompanied by secondary disturbance of adjacent epiblast. He characterizes the lesion as of the nature of an acute infective granuloma. He found that the histological response is of the same nature when inoculation is made into mesoblastic tissue like the spleen as into the epiblast, and considers, therefore, that Levaditi and Nicolau's views (1923) that vaccinia manifests a specialized and definite affinity for epiblast is not justified.

The theory that the reticulo-endothelial system possesses a special affinity for the variola-vaccine virus is supported to some extent by blkocade experiments with indian ink and possibly other material, for instance, those of Ledingham (1926^2), in which intracutaneous injections of vaccine superposed on areas previously or simultaneously blockaded with ink fail to develop, owing, it is suggested, to previous engorgement of the reticulo-endothelium with particulate matter, though other factors influencing the activity of the virus cannot be excluded. It has been shown by Gins and Weber (1916) and others, that the virus is rapidly absorbed from the blood-stream, but Hoen, Tscherktow and Zipp (1926), Goldman (1928) and others, by means of injections of indian ink blockading the reticulo-endothelial system, have found such absorption thereby precluded, leaving the virus still free in the blood-stream. On the other hand, Gildemeister and Heuer (1927) state that from cutaneously vaccinated rabbits they have recovered the virus from the blood in 19 out of 22 rabbits, from 2 hours after the time of inoculation up to the seventh day ; blockading with indian ink made no difference in the result. There seems no doubt, however, that the reticulo-endothelium plays a very important part not only in harbouring the virus, but in providing, almost certainly in the natural disease (variola) and probably in the artificial disease, in the dermis the site of initial attack. From that point, however, strong predilections are shown for involvement of the epidermic epithelium.

Alastrim.

Smallpox is well known to show great variation in symptomatology and virulence, at one time exhibiting a violent toxæmia, speedily fatal, at others an attack so mild that no eruption is displayed or one so slight that the disease may escape recognition ; between these two extremes many different types varying in degree and intensity are classified. Such variation is no new feature ; it was clearly recognized in pre-vaccination times and again in the early days of vaccination by such men as Jenner (1798), Adams (1807), Marson (1866) and others. McVail (1919) points out that in London in the epidemic of 1901–2, the fatality rate was 16·8 per cent., whilst in the provinces from 1902 to 1905 the figure was only 5 per cent., in some instances as low as 2 per cent., and that this represents a milder epidemic type than had been known since reliable statistics were available.

For some years a mild disease of a somewhat anomalous nature has been from time to time reported in many countries. It was first noted apparently in the West Indies in 1865, then in South Africa, where it has been declared to have been prevalent in 1876 (Fehrsen, 1922), in Brazil in 1910 by Ribas (1910) and Australia in 1913. This disease has come much into prominence in England and Wales during the last ten years. It has received various local names such as amaas from South Africa, alastrim from Brazil, Cuban itch, and in this country such designations as variola minor or para-variola. The term alastrim is now well known and is used here for the sake of convenience and brevity. Its first appearance in this country was at Beccles, where a small outbreak occurred of 31 cases, which was investigated by Copeman (1920), who discussed the salient features and differential diagnosis from true smallpox and varicella. The disease is clinically like smallpox, but smallpox shorn of its serious features. In that respect it resembles chicken-pox, for which it is often mistaken. The mortality from it is practically nil. The question has, therefore, arisen whether the disease is in reality smallpox, a variant of smallpox, or a distinct disease having a definite entity. A good review of the subject is given by Tomarkin and Carrière in Kolle and Wassermann's Handbook (1913), and more recently the identity, epidemiology and differential diagnosis have been ably considered by Jorge (1924).

Clinically a few discrepancies in the signs and symptoms have been noted, suggesting a differentiation from smallpox, but on the whole the resemblances predominate. The incubation period appears to be the same. Some have found it slightly longer or shorter than the rigid 12 days usually assigned to classical smallpox. Such variations are common in outbreaks of smallpox; moreover, the difficulties of accurate estimation of this point are well known. The onset may be either abrupt or gradual. A distinction from smallpox has been claimed on the ground that, according to some observers, the onset is commonly gradual. Ricketts (1908) asserts that this is generally the case in variola vera. Prodromal or toxic symptoms may be absent, on the other hand, they may be well marked. Toxic rashes are rare, as also are hæmorrhages, but they do occur. They are not common in normal smallpox, and Ricketts (1908) in an ordinary epidemic puts their occurrence at not more than 1 in 10. Hæmorrhagic types and hæmorrhagic eruptions have also been occasionally observed; they are generally fatal.

The focal eruption. It is generally admitted that the rash shows the same distribution as in smallpox. Similarly, it commonly appears on the third day, but sometimes there is delay, and it may not be apparent till the eighth day, or even later. Delay till the seventh day is occasionally met with in smallpox, but beyond that it is certainly rare; if such an intermission is common in this mild smallpox, it is worthy of note as a point of distinction. The efflorescence goes through the same stages of macule, papule, vesicle and pustule; but it is said that the lesions are somewhat smaller and more superficial than in smallpox. In shape some appear as the usual flattened convex showing umbilication, others, and according to some observers these predominate, are dome shaped, unilocular, and show no umbilication, but vesicles of this configuration are well known to occur in smallpox. Involvement of the mucous membranes of throat and respiratory passages may also occur and sore throat is a fairly common symptom.

With the appearance of the focal eruption there is a return to a feeling of health, as in smallpox, but in alastrim this feeling continues throughout the disease, it is not ended by the occurrence of the secondary fever as in smallpox. Development of the exanthem is apt to be more rapid and the lesion dries up and becomes a crust by the end of the first week of the eruption. In other cases pustulation may occur and slight secondary fever, but commonly incrustation proceeds without further constitutional disturbance, followed in a few days by scabbing. Pitting may or may not occur depending upon the severity of the lesions. The whole process bears a considerable resemblance to the description of variolation, and also to varioloid or smallpox modified by vaccination.

The salient features of alastrim, then, are that it is generally exempt from the secondary fever and the suppurative stage, though these can by no means be entirely excluded, and that the general condition of the patient bears little relation to the amount and extent of the rash. The minor discrepancies that have been noted are all to be found in true smallpox, though in alastrim some of them seem to be the rule rather than the exception.

Further research is needed to ascertain whether the streptococcus has the same prevalence and plays the same role in both forms. It seems probable that in alastrim there is not that association with the streptococcus which is such a frequent characteristic of true smallpox even in its earliest stages.

Experimental Investigations into the Nature of Alastrim.

Paul's test. Material from skin lesions of patients suffering from alastrim has been tested in numerous cases. Certain observers have failed to obtain a due proportion of positive results, but on the whole the same success has attended its use as that of material from cases of undoubted smallpox.

Human allergic test. Tièche (1924) in allergic tests on himself and others found that Swiss alastrim gave the same reaction as material from cases of severe smallpox, but with material sent him from the epidemic in Ponta Delgada he had doubtful results in 4 cases out of 6, 1 typical and 1 weakly positive. The writer applied the test to himself on one occasion and obtained precisely the same result as he ordinarily does with vaccine lymph, namely, some itching commencing about 4 hours after the inoculation, followed by a slight mount and redness, which after 24 hours gradually fade away.

Animal allergic test. Leake and Force (1921) investigating alastrinic material from the outbreak in the West Indies of 1920 found that rabbits previously immunized to vaccinia gave positive intracutaneous reactions with the material, but remained negative to varicella. Similarly, Blaxall

(1923) obtained positive intracutaneous reactions on calves and rabbits previously immunized to vaccinia with alastrinic material in every instance, whether boiled or unboiled, and in some cases to a dilution of 1 in 1,000; the reactions were of the same character and intensity as those given by vaccine lymph or material from cases of undoubted smallpox. With varicellar material treated in the same way no reactions were obtained.

Animal inoculations. As with true smallpox, inoculation of calf, rabbit or guinea-pig with alastrinic material is seldom successful at the first attempt; a slight redness at the site of inoculation and a little desquamation is all that results. It is possible, however, by repeated passage of this slender desquamatory material to arrive eventually at definite vesiculation. Monkeys are freely susceptible and respond in the same way as with variolous material, a vesicular eruption being produced on the fifth or sixth day, which is in precise conformity with that produced by the inoculation of true smallpox material or with vaccine lymph.

Cleland and Ferguson (1915) inoculated six calves with material from the outbreak of very mild smallpox in New South Wales. The primary results were almost negligible, but by transference at the third and fourth removes they obtained definite vesiculation indistinguishable from that of vaccinia. Green (1915) using similar material sent to him in London obtained on calves somewhat equivocal results, and by transference was unable to obtain typical vesiculation. Green failed also with guinea-pigs, using a large series and inoculating them on the scrotum. In the inoculation of eight monkeys, however, with the same material he obtained definite and typical vesiculation at the first attempt in each case. Cross immunity experiments on monkeys gave inconclusive results, the number of experiments being small. Leake and Force (1921) with material of West Indian origin produced on monkeys regularly a vesico-papular eruption similar to that obtained on monkeys inoculated with ordinary variola. They also obtained definite cross immunity on monkeys between alastrim and ordinary smallpox and alastrim and vaccinia. Inoculation of rabbits with alastrinic material gave no definite results, but the rabbits were found subsequently to be partially immune to vaccinia. This immunity was similar to that shown by rabbits inoculated with smallpox obtained from the second remove on monkeys.

Blaxall (1923) investigated material from 12 cases of the mild smallpox or alastrim prevalent in the North of England, comparing it at the same time with material obtained from a virulent outbreak at Poplar (24 deaths in 74 cases). Samples inoculated on calves and rabbits gave no result, but inoculation of monkeys produced in each instance, on about the fourth day, papules which, in the course of a day or two, became definite and typical vesicles. The eruptions produced by the material from the two sources appeared in all respects alike, and similar to that produced on the monkey by inoculation of vaccinia. The vesicular material collected from the monkeys from either source and transferred to calves and rabbits produced no effect at the first attempt, save a slight redness and desquamation. By carrying this material on, however, definite vesiculation resulted both in calves and rabbits at the third remove. Animals in which such vesiculation was produced were found subsequently to be immune to vaccinia. Monkeys which had received the primary inoculation of smallpox material or alastrim were also found to be immune to vaccinia, and animals previously vaccinated were immune to the smallpox material from either source. Van Hoof (1923), Gordon (1925), Turkhud and Pandit (1926) also succeeded in inoculating monkeys with alastrinic material.

Ledingham (1925) reviewed the experimental side of the question, and concluded that the evidence was sufficient to establish the essential identity of alastrim and variola.

Recently Sobernheim and Zurukzoglu (1928) investigated material from mild smallpox cases occurring in England. They inoculated rabbits cutaneously; no definite result was obtained on the first rabbit, but by the repeated passage method at the fifth remove (in a second experiment at the third remove) typical vesicles developed. Carried on to calves these vesicles produced a vaccine indistinguishable in character or potency from ordinary vaccine lymph. Reciprocal immunity was demonstrated between alastrim and vaccine lymph.

Serological Tests.

Gordon (1925) found that material from cases of alastrim and from cases of severe variola alike gave the complement-fixation and agglutination tests to anti-vaccinia serum prepared from the rabbit. Some doubt, however, has been thrown on the specificity of these reactions, the possibility of the presence of concomitant bacterial antigens not being excluded—Schultz, Bullock and Lawrence (1928). Sobernheim demonstrated that anti-vaccinia serum had the same virulicidal action on alastrim of English origin as on vaccine lymph. Okawachi (1924), Schneider (1923) and others have shown the same action in regard to the sera of patients recovering from alastrim of Swiss origin. These sera possess virulicidal antibodies having the same effect as those of vaccine serum or severe smallpox serum. More recently Burgess, Craigie and Tulloch (1929) have used the agglutination or flocculation test for the diagnosis of cases in an outbreak at Dundee with marked success.

Such results as these leave little doubt but that the virus of so-called alastrim in its ætiological and immunological relations is indistinguishable from that of typical variola. Clinically, however, there appears the difference that in typical smallpox the virus has the power of generating a toxin which appears to conduce to the growth and morbid activity of the pyogenic organisms, especially the streptococcus, either by direct stimulation or by hampering or restricting the normal bacteriolytic action of the tissue cells, which would otherwise prevent the development of these organisms. In alastrim such powers appear to be almost entirely in abeyance. Alastrim, in fact, appears to be smallpox deprived of the greater part of its toxicity and divorced from streptococcic commensalism.

Immunological Relations.

It is generally agreed that Jennerian vaccination protects against this mild form of smallpox or alastrim. The incidence of the disease is almost entirely on the unvaccinated, and where the vaccinated are attacked it is on those who have outworn their primary vaccinal protection. It has been demonstrated in regard to the outbreak in England and Wales,* that, of 10,017 cases of smallpox occurring in 1926, 7,956 were unvaccinated and of these 4,840 were under 15 years of age. 2,061 had been vaccinated in infancy, and of these only 8 were under the age of 15 (3 aged 13 and 5 aged 14), and during an epidemic in Switzerland,† there were recorded 4,576 cases of which 4,119 were unvaccinated. Exceptions, however, have been recorded in which an attack has occurred in spite of recent vaccination. In Algeria, Lemaire (1926) states that the protection afforded by vaccination is sometimes particularly brief: of 70 cases of smallpox which occurred in children under 7 years of age, in 16 the attack occurred within one year of successful vaccination.

Alastrim also confers immunity against vaccination in the great majority of cases in which it has been tried, although exceptions are somewhat more numerous. Probably the reaction depends to some extent on the interval that has elapsed between the vaccination and the illness, and also on the severity of the attack. It is well known that successful vaccination is not infrequent after comparatively recent smallpox, and on the whole the protection conveyed by alastrim against vaccinia seems to be much the same as that conveyed by smallpox. As to the duration of the protection it is too early yet to obtain definite figures, and the same applies to the question whether alastrim protects against itself. So far as our knowledge goes on this point, alastrim appears to give as stable an immunity as typical smallpox, at any rate for some time, for no second attacks have as yet been reported.

Human Variolation.

Human variolation has been practised from time immemorial. The source of the contagion was the contents of pocks or pustules or the dried scabs. The Chinese applied old crusts powdered to the nostrils; the Brahmins in India used preserved crusts and inoculated the skin, generally of the arm or forehead; the Persians ingested prepared crusts. In Europe the practice mostly in vogue was to take fluid from pocks as exhibited in a mild case and to apply this to the skin scarified by needle or lancet, or after the application of blisters. As experience grew, greater care was exercised in the selection of the material used, and it became customary to take clear fluid only from vesicles in the early stage. The application might be in one place only or in as many as five or six, and on any part of the body, from the ankle to the forehead, including the navel. The usual site, however, was on the upper arm, frequently on two or three areas on both arms. A method which found much favour in England was that of Sutton (1763). His procedure was to make an oblique puncture in the skin ' not deeper than the sixteenth of an inch ' with a lancet ' charged with the smallest perceivable quantity of unripe, crude or watery matter ', which was taken from the inoculated site of a patient before the true exanthem appeared. In using material taken from the inoculated site he differed from most of his confrères. Dimsdale (1779), who adopted his method, says that he had taken a little clear fluid from the elevated pellicle on the incised part even as early as the fourth day, ' and it appears very clearly . . . that this moisture is capable of communicating the smallpox with the utmost certainty '.

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* *Rep. Minist. Hlth. Lond. for 1926.*

† *League of Nations Comm.*, CH/Variola/25 (1), July, 1928.

Human variolation in the vast majority of cases resulted from a deliberate attempt to inoculate through a recognized trauma, but occasionally cases have occurred through accidental infection of skin or mucous membranes.

The Course of Human Variolation.

For three days no change was apparent at the seat of inoculation. On the fourth day itching occurred and papular elevations were noticeable on the site. These became vesicular and by the sixth or seventh day had the appearance of typical variolar vesicles with clear contents. At this stage the inoculated area was swollen and inflamed, and the patient became feverish and showed constitutional symptoms. This condition continued for about three days, from the eighth to the tenth day following inoculation. During this period a rash ' like fleabites ' might appear on the body. The temperature then fell and the general condition improved. On the eleventh day the true exanthem usually appeared, the number of pocks varying in individuals from a dozen or two to four or five hundred. These pocks passed through the usual stages, but were rarely followed by any marked degree of suppuration or secondary fever. In this respect variolation resembled varioloid or smallpox in those partially immunized by vaccination. The pocks dried up and separated in the usual way leaving no permanent scars. At the inoculated site scarring was nearly always present.

Comparing this syndrome with that of variola the same three stages are to be noted : (1) an incubation stage, but of 8 days instead of 12 ; (2) an initial or toxic stage of the same duration as in variola, namely, 3 days ; and (3) a stage of focal eruption in which the rash displayed the same formation and character but was generally exempt from suppuration and the secondary fever.

Incubation stage. At the site of inoculation there is an incubation period of 3 or 4 days before any reaction is apparent. Four days later, on the seventh or eighth day, fever and constitutional symptoms manifest themselves. This then appears to be the true onset of the disease, and the incubation stage is reduced from 12 days to 8. The difference is in all probability due to the virus developing in a different way, according as it obtains ingress to the tissues naturally, as in smallpox, or artificially, through a trauma, as in variolation.

Initial or toxic stage. In inoculated variola the toxæmia which is so important a phase in smallpox is not lacking, though much diminished in intensity. Fever and constitutional symptoms were recognized as occurring with considerable regularity from the eighth to the eleventh days distinguishable from the disturbance caused by the local reaction. Vasomotor and angiolytic action are evidenced in the occasional erythema occurring at this period, and in the correspondence of the focal eruption in formation and distribution with that of variola. The difference in toxicity, therefore, appears to be one of degree rather than of kind.

The focal eruption. The exanthem in variolation passes through the same stages as in variola, but suppuration and the secondary fever are usually either absent or slight. This may be attributable in part to the use of an attenuated virus, for mishaps in the way of severe attacks more commonly occurred where little care was taken in the selection of the virus, but probably more to the presence of antibody engendered at the inoculated site.

Vaccinia in Man Compared with Variolation.

The incubation period of the local lesion in vaccinia in man is the same as in inoculated variola—namely, 3 to 4 days, and the development of the pock is the same, beginning with a papule on the fourth day, which becomes vesicular on the seventh or eighth day and matures at the tenth or eleventh. In vaccinia the temperature rises slowly from the fourth day to the ninth apparently in conformity with the development of the local lesion—but there is nothing comparable with the pre-eruptive fever of variolation and above all no general eruption follows. Nevertheless occasionally minor conditions are observed in vaccinia which suggest an analogy with smallpox. For instance, rashes may occur in the course of vaccinia on the ninth or tenth day recalling in some measure the prodromal rashes of smallpox. Occasionally an erythema in the femoral triangle has been observed resembling the bathing-drawers rash. Punctate hæmorrhages also occur, especially in the neighbourhood of the local lesion when this is severe. It seems quite possible that these are due to vestigial effects of the primary toxin of variola. A generalization of the eruption occasionally occurs, the so-called generalized vaccinia, but the analogy with the variolous eruption is not evident. The date of appearance varies much. Acland (1899) says that it does not usually occur before the fourth day nor after the tenth, but later dates have been observed. The pocks continue to erupt day by day sometimes for as much as fifteen days. Fever and constitutional disturbances are as a rule little marked. Such occurrences suggest idiosyncratic failure to acquire immunity rather than a reversion to smallpox type. The eruption has never been known to convey smallpox.

Ætiology.

As there is nothing known which definitely differentiates the ætiological factor of smallpox from that of vaccinia, it is convenient to consider the two conditions together, especially since most of the experimental studies on this subject have been concerned largely with the properties of vaccine lymph.

Attempts to demonstrate a specific organism in smallpox lesions by ordinary bacteriological methods have been numerous, and many bacteria have from time to time been isolated and made the subject of experiment, but to none of them could an ætiological role be definitely assigned.

The fact that the clear contents of a smallpox vesicle, though capable of conveying the disease, might yield no organism recognizable microscopically or culturally, excluded the usual bacterial forms. Grünhagen (1872) appears to have been the first to direct attention to forms present in vaccine vesicles which were suggestive of the protozoa. He was followed by Van der Loeff (1887) who described amœboid bodies in vaccine lymph and in the contents of smallpox pustules. He found similar bodies in the blood of variolous patients, of vaccinated children and of vaccinated calves. L. Pfeiffer (1887–96) made many contributions to the subject. He described an organism—'*Monocystis epithelialis*'—which he found in the epithelial cells of the lesions of variola and vaccinia. In a later communication he described amœboid forms in the blood.

In their histological studies of variolous skin lesions, Weigert (1874) and Renaut (1881) both mention the occurrence of small roundish bodies in the interior of epithelial cells—Renaut looked upon them as parasitic.

Guarnieri (1892) described bodies which he had found in the cytoplasm of epithelial cells of the skin in smallpox and vaccinia lesions. These bodies lie in clear spaces in the cytoplasm and may indent the nucleus. They vary in size from a coccus to that of the nucleus itself. He believed they grew and multiplied by simple fission, and by the formation of sporocysts. Guarnieri used largely the rabbit cornea inoculated with variola or vaccinia as a field for the study and demonstration of these bodies. He named them *Cytorycytes vaccinæ et variolæ* and considered that they were the specific cause of these conditions. Monti (1894) described similar appearances in skin lesions of variola and inoculated rabbit corneæ. Pfeiffer (1895) supported Guarnieri's views with further work of his own, correlating the cell inclusions with the amœboid forms he had previously described in the blood. Jackson Clarke (1894 and 1895) warmly upheld the Guarnierian theory and in a later publication (1921) found, in what he considered to be the development and evolution of the Guarnieri bodies, strong analogies to the malarial parasite and to various sporozoa of animal and plant parasitology. Ruffer and Plimmer (1894) described as cell inclusions similar bodies having amœboid movements in variola and vaccinia. v. Wasielewski (1901) confirmed Guarnieri's work. He considered the bodies to be characteristic of and peculiar to the lesions in variola and vaccinia. He urged that from the inoculated rabbit cornea the virus can be carried on for many generations and be transferred successfully to calf and to children, that the bodies were a constant feature in the lesions produced, and that similar bodies were not found in any other known form of degenerating cell.

v. Prowazek (1905) described minute bodies which he called elementary bodies. These were filterable and might be within or without the cell. He considered that the life-cycle of the virus began and ended with this form. He also described other intracellular bodies which he called initial bodies. These were found in smear preparations from vaccine vesicles and in the inoculated rabbit cornea. He considered that they belonged to the chlamydozoan group and had an ætiological significance.

Strong support for Guarnieri's theory came from Councilman, Magrath, Brinckerhoff, Calkin and Tyzzer (1904), who made an extensive research into the pathology and ætiology of variola and vaccinia. They confirmed the occurrence of the cell inclusions in the lesions of the skin and mucous membranes in variola and vaccinia and in the inoculated cornea. Through their investigations they developed the thesis and described, as well as a cytoplasmic cycle, an intranuclear stage which they regarded as sexual. This stage was peculiar to variola and was not found in vaccinia, a difference in morphological evolution which they considered was borne out by the epidemiological and clinical differences of the two diseases.

Guarnieri's views, however, have met with many dissentients. Ferroni and Massari (1893) found the same appearances in rabbit corneæ injured in other ways, and considered they were products of the nuclei of epithelial cells and leucocytes. Salmon (1897) took the same view though he considered the bodies specific and transferable from animal to animal. Hückel (1898) in a very painstaking research on the subject came to the conclusion that the bodies were not living parasites but specific degeneration products of the cytoplasm of epithelial cells. He agreed that they were inoculable from animal to animal and that immunity was produced. Borrel (1903) took a similar view and noted that like products were to be seen in various other morbid conditions such as clavelée and some of the exanthemata. He regarded the bodies as of leucocytic origin. Sikorsky (1903), with whose views Besredka (1903) appears to agree, London and others also regarded them as not of a specific nature. Other observers, however, considered that the bodies were derived from the nuclei or nucleoli (Babes, 1894; Mann, 1899; Süpfle, at least in part, 1914), a view recently again put forward by Ledingham (1926^3), and also supported by Ludford (1925) in analogous studies on tar cancer.

v. Prowazek argued that the Guarnieri bodies could not be considered the actual parasites, but that they arose as reaction products of the cell plasma due to the action of the virus, which he considered to be the elementary bodies, these forming one stage in the development of the parasite. Gins (1922), on the other hand, considers that it is highly probable that the Guarnieri bodies represent a characteristic stage in the development of the virus. Schütz (1925) in a recent study of the subject returns to the older view and considers that these bodies are true cytoplasmatic structures caused by the virus. He thinks that they have no part in a development cycle, but are expression of various kinds of degeneration.

In this rather unsatisfactory state the matter remains at the present time, but a strong body of opinion is gradually accumulating against the parasitic nature of the phenomenon. With further cytological knowledge, and perhaps the assistance of cultures grown *in vitro*, it seems probable that a solution of this question may shortly be reached.

Various other bodies considered to be of a parasitic nature have been noted and described from time to time by numerous observers, such as Doehle (1892), Roger and Weil (1900), Bosc (1901), Funck (1901), Reed (1897), Dombrowski (1902), Ishigama (1902) and others. Perkins and Pay (1903) in an investigation of variolous skin lesions say that they have seen most of the forms described by the various observers, but on close investigation came to the conclusion that the majority could be identified as of a fatty nature and due to a fatty degeneration of cells. In an examination of the blood no special bodies were found and in no case could they detect amœboid forms or movement. Mann (1899) in his histological examinations of vaccine vesicles described bodies which he designated as Z granules. He thought they had amœboid movement and showed growth. He hesitated to ascribe to them an ætiological role, and alternatively hazarded the opinion that they might be discharged leucocyte granules.

v. Prowazek's elementary bodies have already been alluded to. Casagrandi (1911) described very small immotile bodies in filtrates and corneal sections. Volpino (1909) found in the epithelial cells and also intercellularly in the inoculated rabbit cornea small motile bodies $0{\cdot}2\mu$ in size. Paschen (1906) drew attention to very numerous granules which could be demonstrated in dilute variolous lymph, in child lymph, in the inoculated rabbit cornea and in other vaccinial material by special staining methods. He found similar bodies in varicella, but displaying slight differences in position and appearance. The bodies can be demonstrated in vaccine lymph, especially two-day lymph, which is the vaccinal product on the calf after two days' inoculation, in enormous numbers ; they are small, round, coccus-like in form, about $0{\cdot}2\mu$ in size. They are Gram-negative and are not stained by Sudan or osmium. They are resistant to 2 per cent. potash, 2 per cent. acetic acid, alcohol, ether and chloroform. Their presence has been confirmed by several observers ; the writer has seen them in neuro-vaccine (brain emulsion from an intracerebrally inoculated rabbit). They can be agglutinated 1 in 50 by anti-variolous serum. Paschen considers them specific owing to the constancy of their presence in variolo-vaccine lesions and their absence elsewhere. v. Prowazek (1905), Hallenberger (1917[1 & 2]), Ungermann and Zuelzer (1920) and others have expressed the opinion that probably the Paschen bodies represent a certain phase in the development of the virus. On the other hand, objection has been raised by Huntemüller (1914) and others, that such forms cannot be differentiated from albumin or colloid granules. Nevertheless, it seems probable that these bodies, with which may be associated Weigert's granules, Mann's Z granules, v. Prowazek's elementary bodies and similar forms described by others, constitute some form of the ætiological factor.

The Concomitant Organisms in Variola.

These are mainly pyogenic organisms, *Staphylococcus aureus, albus,* and *Streptococcus pyogenes,* of which the streptococcus is by far the most frequent, most abundant and most important.

Presence in lesions of focal eruption. Hines (1922) examined the lesions in 20 cases and found streptococci in 5, of which 3 were hæmolytic and 2 non-hæmolytic. *Staphylococcus albus* was found alone in 7 cases and twice in conjunction with streptococci ; in one of the cases the staphylococcus was hæmolytic. No streptococci were found in the vesicular stage. Perkins and Pay (1903) examined 30 lesions at all stages and found pyogenic organisms negative in all but 4, the streptococcus occurring twice. No pyogenic organisms were found in the vesicular stage. The same authors examining the blood of fatal cases within an hour, or at most two hours, of death, found streptococci present in 38 cases out of 40. *Staphylococcus aureus* was found in about one-third of the cases.

Blood examination during life. The same authors examined by this method 13 cases, of which 8 were subsequently fatal, and in 7 of these streptococci were found, 1 *Staphylococcus albus* only. In the remaining cases streptococci were present in 1 only. Arnaud (1900) reported streptococci in 2 hæmorrhagic cases and Ewing (1902) negative results in 10 varied cases. Most observers agree that immediately before death and directly after there is a rapid spread of the streptococcus throughout the body so that post-mortem observations are not comparable with those made during life. Councilman, Magrath, Brinckerhoff and others (1904) lay much stress on the frequency and importance of streptococci. Like others, they seldom found pyogenic organisms present in considerable numbers in the skin lesions. They were only demonstrable in 6 cases of apparently 54 examined and all were streptococci. Streptococci were very numerous in the lesions of the mucous membranes, as Hugenin (1897) had previously stated. They agree with Perkins and Pay that the bronchial mucous membrane is the chief seat of septic infection, and consider that pyogenic organisms are much more numerous in the lesions of the mucous membranes in variola than in lesions of any other disease.

From this brief summary it will be seen that nearly all observers agree that pyogenic organisms do not form a constant or important feature in the skin lesions, their presence is only occasional and their numbers are small, so that as far as the skin is concerned they take but little part in the suppurative fever; on the other hand, the presence and abundance of pyogenic organisms, especially streptococci, in the mucous membranes is highly significant. It would appear that the pyogenic invasion may be due to some change in the normal cellular bacteriolytic function consequent on the invasion by the specific virus or its toxin. In this connection, Thompson (1904) has shown that in the early stages of the disease the normal bacteriolytic complement is diminished, with a return to normal in the cases in which there is no secondary infection, but where this occurs there is a continued diminution of complement. It would seem that experimental research might be directed with advantage to increase bacteriolytic power by the aid of extraneous complement.

Animals Susceptible to Variola and Vaccinia.

Natural Variola.

Monkeys, like men, appear to be able to contract variola naturally. This view rests on statements made by Schmidt (1870) and Bleyer (1922), who states that mild smallpox among the natives of the region of the Upper Uruguay River in Brazil spread to monkeys (*Mycetes* and *Cebus*) whom it killed in large number. Corpses were found under trees and the bodies of sick monkeys as well as dead were covered with variolous pustules.

In regard to other animals there is no evidence that sheep ever contract the disease from man; cows if infected naturally transmute the infection into cowpox (Sonderland, 1831; Ceely, 1840), but there seems some possibility that swine may acquire the disease naturally from human variola, though indirectly (see Chapter IX, Swine Pox).

Variolation.

Monkeys can be inoculated cutaneously with variola and the disease produced is very similar to vaccination in monkeys or men. No apparent change is noticed till after the third day when the inoculated area reddens and shows slight swelling; on the fifth day vesicles appear which come to maturity about the seventh or eighth day, the general appearance being indistinguishable from vaccinia. As a rule constitutional symptoms are not marked, but sometimes the monkey becomes somewhat dull, its eyes may be suffused and there is a slight rise of temperature. In a case recorded by Copeman (1894), on the ninth to the eleventh day a general eruption appeared over the whole surface of the body, as Zülzer (1874), Voigt (1881) and others had noted in other cases. This, though unusual in the writer's experience, is analogous to the course of variolation in man. The exanthem on the monkey, however, differs from that of vaccinia on the same animal in that it is not easily communicable to calves or rabbits. Generally, at the first attempt, there is no sign of reaction, but if the inoculated area be scraped and the debris carried on to another animal and this operation repeated, vesiculation, generally quite typical in appearance, follows at about the third passage. The eruption on the monkey appears to be still variolous, and further passage is required on calf or rabbit to transform it into vaccinia. Whether the monkey is infectious or not it is difficult to say, but in a few instances the attendants on variolated monkeys have contracted the disease, which, however, may have been due to infection from the original material. On the other hand, on two occasions the writer has placed healthy uninoculated monkeys in close contact with variolated monkeys for the whole period of the disease, but they showed no sign of infection and displayed no immunity subsequently to variola or vaccinia.

Besides the monkey, variola can be inoculated on many other animals —the cow, calf and rabbit in particular—but once vesiculation is produced and perhaps carried on for one or two removes the disease apparently ceases to be variola ; it becomes a purely local affection, produces but slight constitutional symptoms, and is not infectious to other animals, in fact, it has become vaccinia. When the change happens, how it is brought about, and to what influences it is due, are problems which are at present unsolved, but the experimental facilities for the investigation of the disease and its ready inoculability lead one to hope that further study will bring elucidation before long.

Variolation of the cow or calf, however, is not readily achieved and it seldom happens that typical vesicles are produced by direct inoculation. Gassner of Gunsberg, in 1807, appears to have been the first to succeed, after ten attempts. He was followed by Thiele (1836), Ceely (1840), Badcock (1840) (who made over 500 experiments, succeeding in only 7 per cent. of them), Voigt (1881), Haccius (1892), Simpson (1892), King (1889) and others. In these experiments milch cows were generally employed: cutaneous inoculations were made on the udder, where definite vesiculation was obtained in only a very small percentage of the attempts made. On the other hand, Chauveau and the Lyons Commission (1865) failed, and only succeeded in carrying on smallpox. Martin (1860) of Boston had a somewhat similar experience. He variolated a cow and carried the lesions direct to children with the result that they contracted smallpox. Klein (1894), using calves, had some success after many attempts, and Copeman succeeded once out of four attempts. It is now recognized that calves can without doubt be inoculated cutaneously with variola, either by the repeated passage method, or, more certainly, through the intermediary of another animal, such as the monkey. Rabbits react in the same way as calves to variolous inoculation : primary vesiculation is rare, but by passage success is obtained, though several passages may be required. Sheep, horses, fowls, have similarly been variolated, and probably all animals susceptible to vaccinia are inoculable with variola. Variolation by other routes has not been systematically studied. Warlomont (1883) described in horses a generalized variolous eruption following intravenous inoculation. Paschen (1911) has shown that rabbits so infected acquire immunity to skin inoculation with vaccine, but after intraperitoneal injection no immunity results. By subcutaneous injection, immunity has been obtained by Warlomont (1883), Béclère, Chambon and Menard (1896), Paschen and many others.

Positive results are also obtained with corneal inoculation. A keratitis is set up with minute elevations on the surface, which, with the recognition of Guarnieri bodies microscopically, is a reaction frequently employed for diagnostic purposes and known as Paul's test. Intratesticular inoculation sets up an inflammatory process, and this carried on cutaneously has occasionally given rise to definite vesicular lesions. Ledingham (1926[1]), McKinnon and Defries (1928) and others have shown that normal rabbits are susceptible to intracutaneous inoculation with variola, the reaction being purely local and of the same nature as that set up by vaccinia. A similar reaction occurs in rabbits previously immunized to vaccinia, but according to McKinnon and Defries the development in these conditions is more rapid and the retrogression takes place sooner. Normal rabbits react much more readily to this mode of inoculation than to cutaneous scarification, a positive result being always obtained intracutaneously, but the resulting lesions do not render the virus more adaptable to the surface skin, rather the reverse. Since, judging by vaccine analogy—according to Ledingham and McClean (1928)—there is a conspicuous proliferation of the virus in these intradermic reactions, there appears to be some factor missing or in abeyance in variola inoculations, which hinders the surface manifestation; this is a matter which requires further investigation. The same observers suggest that anaerobiosis may be a factor, and this seems quite possible.

Susceptibility to Vaccinia.

Most mammals and birds are susceptible to vaccinia inoculated cutaneously. The calf and the rabbit are those most frequently employed. Statements have been made that rabbits inoculated cutaneously do not form definite vesicles. This is incorrect. Typical well-formed vesicles are produced on the rabbit skin similar in every way to calf vesicles. To obtain this result the inoculation must be made very lightly, just deeply enough to make a break in the epidermis. If the trauma is deeper vesiculation is not good, and only a leathery crust may result.

The result of inoculation of susceptible animals is a local affection which does not become generalized and is similar to vaccination of man or calf. Susceptibility is shown also to inoculation by other routes, but here the affection varies according to the mode and site of inoculation. By all routes, however, immunity is produced, though it is much delayed when the corneal route is used (Gins, 1917). Inoculation of the cornea causes a keratitis with minute elevations on the surface, which, with the recognition of Guarnieri bodies, is used, as in variola, diagnostically.

Though cutaneous vaccination with normal lymph does not lead to generalization, this can be effected experimentally in several ways. Calmette and Guérin (1901) showed that after intravenous injection of normal cutaneous vaccine a general eruption may result, especially on areas of skin previously shaved and irritated. This is by no means a constant reaction, however, and frequently no generalization occurs (Gins and Weber, 1916; Watanabe, 1924; Ledingham, 1924, and others). Camus (1918) was the first to show that intravenous injections of normal cutaneous virus in adequate dose gave rise to typical vesiculation on the skin and marginal mucous membranes of all experimental animals, including monkeys, cats and dogs. He did not note any visceral lesions. Camus's work was confirmed by Uhlenhuth and Bieber (1923) and Watanabe (1924). The experimental use of more virulent strains, such as neuro-lapine, neuro-testicular lapine, &c., in considerable dosage brought about further developments. Levaditi and Nicolau (1923[3]) after intravenous injection of neuro-lapine described similar appearances. Winkler (1927) showed that after inoculation in various ways with similar material, the virus could be recovered from most of the internal organs, especially from the adrenals, but after cutaneous inoculation it was not demonstrable in the brain. Douglas, Smith and Price (1929) using full doses of neuro-lapine and neuro-testicular lapine by the intravenous and other routes found similar appearances to those described by Camus, and also described macroscopic lesions in most of the viscera. Similar results have been obtained by Paschen (1927, 1928), McIntosh and Scarff (1929), Bijl and Frenkel (1929) and Ledingham and Barratt (1929), the last authors giving an histological account of the lesions.

Intratesticular inoculation can give immunity and also set up sympathetic vaccinal lesions in the other testicle if that be injured. Some investigators have found the testicle a more delicate test for the presence of vaccinia virus than either skin or cornea. Repeated testicular passage can increase the virulence so that the virus applied to the skin gives not only a cutaneous eruption but a generalization (Ohtawara, 1922), or injected intracerebrally sets up a fatal encephalitis (Levaditi, Harvier and Nicolau, and others). According to Duran-Reynals (1929) extract of normal testicles enhances markedly the infectivity of both neuro and dermal strains of vaccine virus, kidney extract also but to a less degree; on the other hand, spleen, blood, &c., act as repressors. The tissue extract appears to affect the cells of the host rather than the virus.

The intracerebral inoculation of rabbits by Marie (1920), Levaditi, Harvier and Nicolau (1921), which, under certain conditions, led to encephalitis, demonstrated that the virus could multiply in the brain, and could be obtained in quantity free from other organisms. This neuro-vaccine has been used for human vaccination in Spain with considerable success, but a reasoned estimation of its value for this purpose has not yet been arrived at. Since experiment has shown that neuro-vaccine, neuro-testicular vaccine, &c., have a pronounced tendency to generalization of the virus throughout the body tissues, the use of these forms of vaccine for human vaccination seems inadvisable.

Filterability of the Virus.

Since the specific cause of variola and vaccinia is not resolvable by ordinary microscopic and cultural methods it has been assumed that the virus is ultra-microscopic and a filter passer. Numerous attempts at filtration have been made, but so far the results obtained have been inconstant and equivocal. The first filtration experiment indicative of success was that of Nicolle and Adil Bey (1906), who mixed diluted vaccine with an alkaline solution of pancreatin. The digested lymph was filtered through paper and a Berkefeld candle, the filtrate concentrated *in vacuo* and tested on a calf, with a positive result.

Negri (1905) after a process of maceration in water obtained success after passage through Berkefeld V on a child, calf, and rabbit cornea. Casagrandi (1905) using a negative pressure of one atmosphere passed the virus through a Chamberland F and Berkefeld candles V, N and W, and inoculated a dog and rabbit cornea successfully. He failed at first with Chamberland B and other filters, but succeeded later. Carini (1906), adopting Negri's method, claimed success, as also have van der Kamp (1914), von Prowazek (1912), von Wasielewski (1901), Paul (1919), Gins (1914), Paschen (1913), Hammerschmidt (1919) and others. In nearly all the attempts recorded success has been inconstant and partial, only a small portion of virus passing the filter. Reconcentration of the filtrate is necessary. Von Prowazek for this purpose employed an ultra-filter of filter-paper coated with collodion and 3 per cent. agar, and claimed a larger measure of success by its use. Gins (1914) considers it essential to use highly diluted vaccine for filtration and to reconcentrate. It has been shown by Levaditi and Nicolau (1923[1]) and others that kaolin, animal charcoal or kieselguhr possess the property of adsorbing the virus, and it is suggested that retention in this way may be a possible explanation of the larger part of the virus failing to pass the filter. After shaking diluted lymph with kaolin or kieselguhr, and centrifugalization, the virus can be found in the sediment whilst the supernatant fluid is quite inactive (Kraus, v. Eisler and Fukahara, 1909). By again shaking the sediment with weak ammonia solution (Herzberg, 1925), a part of the virus can be set free. This method has been used in combination with filtration and with the ultra-filter by Gins, but with only occasional and partial success. Though success of a kind has thus been obtained from time to time by different observers there is a freakishness about the results that renders them far from convincing, and it cannot be said that the sum of the achievements is satisfactory. It is significant that there is a wide variation in the porosity of the filters employed, where some have obtained bare success with a Berkefeld V, others have claimed positive results with so dense a filter as Chamberland B. Such discrepancies no doubt may be due to difference in the adsorbing power of the components of the filter, but even so there is a lack of grading according to the density of the filters used, which at present is inexplicable.

Many workers, probably the majority, have entirely failed to prove the filterability of the virus (Thomson, 1924; Ledingham, 1926[1]; Perkins and Pay, 1903; Gordon, 1925; Findlay, 1928, and many others). The conditions which would seem most conducive to success are to bring the vaccine material into as fine a state of division as possible, so that the virus may be evenly distributed through the fluid, to use it well diluted, to remove as far as possible by centrifugalization and repeated washings all adventitious albuminous material, and to reconcentrate the filtrate. The writer has tried to establish these conditions in very many experiments, and has employed every conceivable method, but always without success, though the control has been fully positive.

Further it is not clear what part the supposed virus, as microscopically identified (Guarnieri bodies, Paschen bodies, &c.) plays in this question of filtration. Many of the forms described would scarcely seem to be of a filterable nature or size.

The matter becomes more incomprehensible since Levaditi and Nicolau (1923[2]) have claimed that they have succeeded in transmitting vaccinia virus through a collodion filter of such a nature that it is only permeable to peptones and bacteriophages, resisting the passage of albumins. Bland (1929), however, is unable to confirm this and finds that the virus does not pass 3 per cent. collodion, whether plain, serum-treated, or virus saturated. On the other hand, Ward (1929) claims that an active filtrate can be obtained by triturating with pyrex glass, emulsifying in hormone broth, and passing through a Berkefeld V filter. The writer after several attempts has not been able to confirm this.

It follows that the question as to the filterability of the variolo-vaccine is by no means settled, but a collateral factor in favour of filterability is found in the fact that the viruses present in the various animal pock diseases have been reported as filterable, though the evidence advanced, as in the case of variolo-vaccine, is not always convincing. It may be that the virus in its life-history passes through different morphological changes, and that in only one phase is it filterable, and that perhaps an immature and partially inactive phase. The solution of the problem seems hardly likely to be reached until pure cultures of unassailable integrity are available for experimentation.

Action of Physical and Chemical Influences on the Variolo-Vaccine Virus.

Physical Influences.

Dryness. Variolous crusts retain the power of communicating the disease for years after removal from the body. The main conditions for the preservation of virulence are dryness and non-exposure to heat. The vaccine virus also withstands artificial drying as shown by Blaxall (1902). Dried *in vacuo* over sulphuric acid or calcium chloride it retains its potency for at least four months at room temperature, and, as further experience has shown, for years in cold storage; also for a considerable time at 37° C. (Tomarkin and Serebrenikoff, 1910). Green (1908) found that dried vaccine withstood 100° C. for 5 to 10 minutes.

Heat and cold. Vaccine lymph freshly collected from the calf and mixed with water withstands a temperature of 57·5° C. for 5 minutes without loss of virulence but 60° for the same time is lethal (Blaxall, 1902). Gordon (1925) using dilutions of glycerinated lymph and variolous crusts in saline found 55° C. for 30 minutes the lethal point for the latter, but not completely fatal for the vaccine.

Variola virus and more especially vaccine virus can be preserved in glycerin either pure or in dilution. It is essential, however, that the glycerinated virus should not be subject to the influence of heat, for the germicidal action of glycerin on vaccine varies directly with the temperature (Blaxall, 1904). Green (1908) showed that the fall was in accordance with the Arrhenian law expressing temperature effect on chemical action. To obviate this hostile influence of glycerin on vaccine virus experiments were commenced in 1900 on the influence of temperature below the freezing-point (Blaxall and Fremlin, 1908). They found that glycerinated lymph retained its full potency after exposure to the temperature of liquid air, —180° C., for 11 weeks and subsequent experience has shown that at —10° C. no appreciable reduction in activity is observed in 4 years.

Pressure. Vaccine virus withstands a pressure of 300 to 400 atmospheres with impunity (Santori, 1904), and an inoculated cornea subjected to a direct pressure equivalent to 3,000 ergs retains its infective power (v. Prowazek, 1906). Fremlin (1912) showed that air, or oxygen, at a pressure of 50 atmospheres, acting for 3 weeks, had no deleterious action on vaccine lymph, but that carbon dioxide at the same pressure inactivated it after a week.

Light. Direct sunlight is inimical to the virus, but in this connection the effect of heat must be taken into account. Diffused daylight gradually weakens the activity of glycerinated vaccine. Exposure to ultraviolet rays for 30 minutes destroys the adventitious bacteria, but has no appreciable effect on the virulence of the vaccine virus. Sporing micro-organisms were affected sooner than the vaccine virus (Friedberger and Mitronescu, 1914). Finsen light acts more rapidly, Mayerhofer (1916), using a quartz lamp, found the virus completely destroyed in 5 minutes.

Radium causes a gradual deterioration in vaccine lymph dependent on the length of the exposure. Poppi (1925) and Muttermilch and Ferroux (1925) found the same in regard to neuro-vaccine.

Chemical Influences.

Green (1902–4) found resistance greater to an inorganic alkali (sodium carbonate) than to an inorganic acid (hydrochloric acid). Of disinfectants the virus resisted sodium biborate (saturated), perchloride of mercury 1 in 500,000, sodium salicylate 1 in 10, quinine hydrochlorate 1 in 900, boracic acid (saturated), carbolic acid 1 in 500 to 1 in 50, lysol 1 in 500, and chloroform 1 in 200, but succumbed to potassium permanganate 1 in 800, iodine, ethyl alcohol, ether 1 in 10 and toluol. Other substances showing less action on the specific virus than on the extraneous germs were eucalyptol, peppermint, saccharine, glycogen, &c.

v. Prowazek (1906) found that sodium taurocholate weakened the virus and Friedberger and Yamamoto (1909) reported that bile destroyed it.

Oil of cloves was found by Blaxall (1912) to have no injurious effect on the specific virus of vaccine lymph in the strength of 0·1 per cent., but to exert a marked eliminating influence on the adventitious bacteria. Other substances having a like effect are eucopinotoxinum hydrochloricum (Kirstein, 1919), chinosol (Seiffert and Hüne, 1913), brilliant green (Myer Coplans, 1922), trypaflavine (Illert, 1921), and ether (Fornet, 1913[1, 2 & 3]). Gordon (1925) lays stress on the virulicidal action of potassium permanganate, which in some instances, at a dilution of 1 in 100,000, completely inactivated vaccine lymph, and notes that similar results were obtained with variolous and alastrinic materials.

Glycerin. The viruses of variola and vaccinia are more resistant to the influence of glycerin than are the generality of non-sporing bacteria, a characteristic which they share with those of other diseases such as rabies, herpes, foot-and-mouth disease, &c., all of which are filter passers. That glycerin might be mixed with vaccine lymph without detriment has been known for many years. Copeman (1898) records that Cheyne used it in 1850. Müller (1866), in Berlin, employed glycerin in connection with vaccine lymph simply as a diluent. Copeman (1891) was the first to point out that glycerin possessed a valuable selective action in eliminating the adventitious germs, whilst under suitable conditions it exerted no appreciable effect on the specific virus. Chambon and Menard (1892) corroborated this, as also did Leoni (1894). Further experiments were made by Copeman and Blaxall (1898) demonstrating amongst other points the advantage of glycerin over vaseline or lanoline as a vehicle for vaccine lymph. Glycerin may be used pure or in dilution, 50 per cent. in distilled water or saline being the strength commonly employed. Dilutions of 40 to 80 per cent. show little appreciable difference in action. The relation to heat has been already dealt with.

A striking and important difference in the resistance of variola virus and vaccine virus under natural circumstances is shown in their behaviour whilst in contact with the body tissues. In vaccinia of the calf the virus contained in the vesicular scabs whilst attached to the body begins to lose its vitality from the eighth day and shows a progressive deterioration to the fourteenth or fifteenth day, when it is completely inactive and probably dead (Blaxall and Fremlin, 1904). This loss of activity coincides with the production of immunity in the calf. In variola there is no such inactivation, the dried scabs on the body and the seeds in the feet and hands retain their vitality though attached to the body for a considerable time ; it may be two months or more before all the seeds are separated. It is this difference which is at the root of infectiousness. In an infectious disease the virus, though present in the tissues, resists the action of the antibodies, and on separation can disseminate the disease under suitable conditions for prolonged periods—scarlet fever is another case in point ; in a non-infectious disease, on the other hand, the virus perishes *in situ* or almost immediately after leaving the body, and consequently there is no dissemination of the disease.

Artificial Culture of the Virus.

For many years numerous attempts have been made to isolate and cultivate the virus of variola and vaccinia *in vitro*, but until recently with no success. In the early days cultures of cocci and bacteria were claimed to be those of the specific organism, but none received confirmation. It is an essential condition that the virus should be obtained in a pure state, that is, uncontaminated with bacteria, and yet retain unimpaired potency. Fornet (1913) claimed to have realized this condition by shaking calf lymph with ether, and through subsequent culture in suitable media to have grown the organism. His results, however, were not confirmed (Paschen, 1913; Gins, 1913; Harde, 1915; and others). Pröscher (1915) used anaerobic cultures containing ascites fluid, an animal serum and fragments of tissue, kidney or liver, and claimed success on the rabbit cornea up to the twentieth subculture. This again lacks confirmation.

Plotz (1922) used for inoculum the blood of previously vaccinated rabbits, cultivating in Smith-Noguchi medium containing fresh pieces of rabbit kidney; in subcultures the composition of the medium was slightly altered. He claimed success on the rabbit skin and cornea with the fifth subculture. Paschen (1924[1]) and Yokota (1923) have been unable to confirm this. Urbánek (1926), using much the same method, also reported success. On the other hand, Gins (1914, 1916) and Steinhardt and Grund (1915), trying numerous methods, obtained only negative results. They came to the conclusion that the vaccine virus under suitable conditions might live for a considerable time in various culture media, but that there was no evidence of multiplication. Improvements in the art of growing living tissue outside the body have led many investigators to employ this medium for the cultivation of the vaccine virus. Steinhardt, Israeli and Lambert (1913), Harde (1915), Parker and Nye (1925), Craciun and Oppenheimer (1926) have used this method with a certain measure of success. Parker and Nye, using testicular tissue, succeeded in carrying the virus to the twenty-fourth subculture. It was much weakened, however, in potency and gave no reaction on rabbit skin or cornea. Positive results could only be obtained by testicular inoculation. Carrel and Rivers (1927), following the same path, claim more satisfactory results. They used as inoculum testicular material from a rabbit inoculated into the testes with vaccine lymph, diluted it with Tyrode solution and inoculated into the solid coagulated plasma of a fowl together with embryonic tissue of a chicken suspended in Tyrode solution. Every few days the culture was washed with Tyrode solution and nourished with the diluted embryo suspension. At the end of eight days the culture showed a marked multiplication of virus as tested on the rabbit. Levaditi (1927), using chicken embryo and hen plasma with neuro-vaccine, confirmed these results. Eagles and McClean (1929), repeating Carrel and Rivers' method, also obtained success. Maitland and Maitland (1928), using similar testicular inoculum, cultivated it in hen-serum to which were added fragments of hen-kidney with Tyrode solution, that is, apparently apart from living cells. In four successive cultures they claim to have increased the vaccinia virus approximately 25×10^6 times. Rivers, Haagen and Muckenfuss (1929), however, do not agree that the increase occurred in the absence of living cells.

It is early yet to say that these methods offer a solution of the problem of cultivating the vaccinia virus outside the body, but there is every reason to think that they indicate emphatic progress. It remains to be seen what powers of endurance the virus thus cultivated possesses; at present there seems reason to think that under these conditions there is rapid loss of activity. There is, however, certainly a prospect that by means of some such method it may be possible to cultivate the virus on a considerable scale, and if this can be achieved with constancy and maintenance of virulence, a biological advance of great importance will have been attained, for vaccination can then be carried out from a pure culture; moreover, the economic value will be appreciable since it will be possible to dispense with animal production. Apart from these advantages the attainment of a pure culture will go far to elucidate the baffling problems of the morphology and filterability of the variolo-vaccine virus.

Immunity to Smallpox.

Immunity from Smallpox, Variolation and Vaccinia compared.

All people at all ages and of all races are naturally susceptible to smallpox. Exceptions have been noted occasionally, but it is doubtful if this refractoriness is a genuine and permanent condition. In the same way all, provided they have not suffered from smallpox, are naturally susceptible to vaccinia. Cory (1898) states that out of 61,000 primary vaccinations he only met with one case that failed at the second attempt.

Immunity to smallpox may be acquired by a previous attack, by variolation, or by vaccination and revaccination. An attack of smallpox confers immunity which in the great majority of cases lasts for life, but numerous exceptions have been noted. De la Condamine put liability to a second attack at 1 in 50,000, Heberden 1 in 10,000, and Eichorn 1 in 200. Huguenin (1897), analysing the exceptions, stated that the duration of immunity to variola could vary from two to sixty years.

In like manner variolation efficiently performed gave protection as secure as smallpox itself, subject to similar exceptions. Primary vaccination, which Jenner originally thought would give lifelong immunity, is now known to protect only for a limited and variable number of years, which may be roughly estimated at between five and ten. The duration and extent of the immunity produced depends on the efficacy of the vaccination, on the virulence of the smallpox to which the vaccinee is exposed and on the idiosyncrasy of the individual. With the lapse of time immunity is a diminishing factor, and the severity of the disease sustained is in direct proportion to the residual immunity which even to the end of life nearly always suffices to effect some mitigation. The last evidence of a waning immunity is the suppression or determent of the suppurative stage. With revaccination performed at a suitable age such mitigation is very pronounced, so much so that a fatal attack of smallpox in a revaccinated person is rare even though a long interval has elapsed since the second vaccination. That vaccination protects against smallpox was fully established by the very numerous unsuccessful attempts to variolate after vaccination, made by Jenner (1798), Sacco (1801), Pearson (1802) and many others. Sacco carefully investigated the reaction and showed that during the first five days after vaccination, variolation produced only a local pustule with slight general disturbance, thereafter day by day there was a dwindling response till the eleventh or thirteenth day, when generally no reaction followed. Thus vaccinia produced partial immunity against variolation in five days, which was complete about the twelfth day. It is interesting to compare this with the behaviour of vaccination during an attack of smallpox. During the incubation period the vaccinal reaction is good, but with the onset of the disease there is a rapid falling off, and by the second or third day of the focal eruption, or even earlier, vaccination elicits no response (Huguenin, 1897; Ricketts, 1908; Koyamada, 1929). This immunity against vaccinia at such an early stage is remarkable and will be referred to later.

Vaccination gives immunity against vaccinia as Cory showed by day by day vaccination. Immunity was produced on the ninth day, and this was a very definite and constant feature. He also pointed out that the development of the vesicles produced by the subsequent inoculations was greatly accelerated, all maturing at the ninth day, whatever day the vesicle was inoculated. This phenomenon, for which there is at present no adequate explanation, was known and recorded by Bryce (1809), and it had also been observed with the lesions produced in variolation. Cory also found that if supernumerary fingers were vaccinated and the finger removed before vesiculation had appeared—as, for instance, on the second or fourth day—subsequent vaccination ran a similar accelerated course, the vesicles maturing on what would have been the ninth day counting from the date of the original inoculation on the supernumerary finger. In these experiments he demonstrated that partial immunity was set up without the production of vesiculation. When these results are compared it is seen that vaccination gives immunity against vaccination on the ninth day and against variolation on the twelfth day, but in smallpox, immunity against vaccination is produced on the fourth or fifth day of the disease, a noteworthy difference which suggests that the natural disease evokes antibody much more rapidly than when the inoculation is artificial and traumatic.

In animal experiments the reciprocal immunity of variola and vaccinia is not so absolute nor so well defined. Blaxall (1923) found in monkeys reciprocal immunity of variola and vaccinia to be equal in degree and indistinguishable one from the other, but Gordon (1925) found that vaccinia gave better protection against variola than the converse, and Wurtz and Huon (1916), Brinckerhoff, Tyzzer and Councilman (1905–6) considered that variola gave but uncertain and less durable immunity against vaccinia.

Immunity from Vaccination and Revaccination.

It is important in clinical work to be able to form an estimate of the amount of immunity present in men after vaccination and revaccination. Long experience and statistical evidence based on the number, size and form of the scars has clearly indicated that these when carefully observed and coupled with the interval that has elapsed since vaccination, can afford a fairly reliable index of the immunity which has been produced and that which remains. Scars, it is true, do not always necessarily represent the true vaccination effect, but in the main they give adequate guidance, and deviations due to abnormalities can be discounted by experience. Some observations made in America by Leake and Thomas (1926) suggested to them that no relation could be made out between existing immunity and scar formation, but the conditions of the observations were open to considerable criticism. Similar doubt has been expressed by others but no definite evidence has been adduced to displace the old established view.

Immediate reaction. A method of practical estimation of the existing immunity has recently been adopted which has undoubted value. This depends on the immediate reaction, which was first observed by Jenner (1798), studied by von Pirquet (1907) and practised and advocated by Tièche and others. It is now recognized officially in the United States Navy.

There are various ways of carrying out this operation, but the inoculation should always be accompanied by an uninoculated trauma to serve as control, so that a comparison may be made between the two. The reaction serves as an admirable gauge of the degree of immunity existing, the earlier and less the reaction the greater the immunity, and conversely. This operation has the great advantage that if the immunity be low the test inoculation will strengthen it, if high there is no discomfort to the patient.

The seat of immunity. It has long been held, and the view is still maintained by Besredka (1922) and his school, Levaditi (1922) and others, that immunity is a cellular product and is especially an attribute of the ectodermal epithelial cells. In recent years, however, it has been shown that the distribution of the virus, both in variola and vaccinia, is much more widespread than was first supposed, and the modern tendency is to regard immunity as general and humoral rather than local and cellular. Raynaud (1877) was the first to show that immunity did not necessarily depend on vesicle formation, that the inoculated spot could be excised before vesiculation was obtained and still immunity or partial immunity evolved. Cory, as already mentioned, demonstrated the same effect, and the experiment has been repeated and confirmed by numerous observers. It has also been shown that the variolo-vaccine virus speedily passes into the blood-stream, and can be found there under certain conditions within a few hours of the inoculation (Ohtawara, 1922 ; Gildemeister and Heuer, 1928). Here it is rapidly fixed, and it has been shown by Wilson Smith (1929), using a fractionation technique, that white blood-cells are probably responsible for this, though platelets cannot as yet be definitely excluded. From the blood-stream it passes into the reticulo-endothelium, becomes generalized throughout the body and can be recovered from almost all the organs. For details of this distribution reference should be made to Sobernheim (1927), Winkler (1925) and others. Winkler (1927) shows that by all routes and with all kinds of vaccine, the range is general and approximately of the same degree. Of especial interest is his observation of the almost constant occurrence of the virus in the adrenals. To detect the presence of the virus in the different organs resort is had to cutaneous inoculation of susceptible animals such as the rabbit, with the production of vesicles either directly or after repeated passage, to intratesticular inoculation, which by some is considered the most delicate test, to intracutaneous inoculation or to corneation. Knowledge of the persistence of vaccine virus in the tissues has been extended by Olitsky and Long (1929), who by cataphoresis (the virus aggregating at the anode) have shown that virus is detectable in tissues in which it cannot be demonstrated by other tests, and that it can be found in rabbits long recovered from cutaneous vaccination.

An interesting observation is that of Plotz (1927), that after cutaneous inoculation of the rabbit the virus spreads into the surrounding skin and may be found 2 to 3 cm. away within 36 hours, the invasion proceeding further and further from the site as time passes until the maximum is reached on the fourth day. On the fifth day when vesicles have formed, the virus can no longer be found in the skin. Extension into the skin increases proportionately with the size of the area inoculated and the dose employed. Plotz was not successful in detecting the virus in the blood, except when the dose and area inoculated were considerable. He argues, therefore, that the invasion of the skin is not by the blood-stream. He found that the cornea became immune only when large doses were employed and large areas inoculated and suggests that the cornea may be used as a criterion of the amount of immunity produced. His conclusion is that the immunity depends on two factors, the dose and the area involved.

The inoculation of a large area implies the use of a larger dose (strength being equal) than that required for a small area, and in the writer's experience area involved is the more important factor.

Ledingham (1928) considers immunity more dependent on dose than area. The experiments of Clearkin (1929) suggest that a minimal dose gives as strong immunity as a potent dose.

Immunity may be produced by all routes, but special attention may be drawn to intracutaneous inoculations, which give promise of being of great value.

Intracutaneous inoculation. The use of this method was first introduced by Novotný and Schick (1910). Groth (1919) and others adopted it for estimation of potency of vaccine on the rabbit, and lately Ledingham (1924) has exploited the method more fully, both histologically and immunologically. It was the use of this method in the first place that led Ledingham to the conclusion that immunity varied directly with the dose employed. A point of great interest in intracutaneous inoculation is that vaccine from a cutaneous source, as from vesicles on a calf, shows after the first intradermal inoculation some difficulty in adapting itself to further inoculations of this kind, but by repeated passage gains strength and *pari passu* loses its cutaneous affinity, so that after several intradermal passages cutaneous vesiculation is difficult and sometimes impossible to achieve. As has already been said variolous material shows a similar one-sidedness; a good reaction follows intradermal inoculation, but no reaction can be induced cutaneously, a matter of great moment, for which at present there is no explanation. Ledingham and McClean (1928) have also shown that by suitable manipulation virus can be obtained from an intracutaneous lesion free from contaminating bacteria.

Immunization by killed or attenuated vaccine virus. Much difference of opinion still exists on the question whether or not killed virus can produce immunity. The majority of the experimenters have employed heat as the lethal agent, but phenol, rabbit-bile, formol, and exposure to radium have also been used. Exposure to 60° C. acting for variable periods from 5 to 30 minutes is the usual method. Henseval (1919) showed that the heat must not exceed 60° C., and that at 70° C. all prophylactic action was abolished. In all the experiments in which immunity has been produced after the injection of heated lymph the degree has been weak and of relatively short duration, and in few or none has conclusive evidence been given that the virus was actually dead. It is questionable if it is justifiable to assume death from failure to obtain a reaction on rabbit skin or cornea. Experience has shown that such an apparently negative response may occur with living virus whose vitality may subsequently be resuscitated. Many failures have been recorded (Gastinel, 1913; Tomarkin and Suarez, 1917; Groth, 1923, and others). Some of the most successful experiments are those of Nakagawa (1924–5), who, by using 'koktoimmunogen', obtained according to the dose and route employed both local and general immunity.

Gordon (1925), using lymph heated to 55° C. for 30 minutes for the most part, but also attenuated in other ways, found that a certain critical dose was required, and that with this by all routes immunity was produced, beginning about the fourth day and lasting for 50 to 100 days. Gordon admits, however, that the heat exposure was not necessarily sufficient to kill the whole of the virus. Hunt and Falk (1927) also claim successful immunization by all routes. On the whole there remains a considerable doubt whether immunity, certainly immunity of any practical value, can be produced by the use of killed virus.

Serology of Variola and Vaccinia.

It is clearly established that the production of immunity in variolo-vaccine affections is attended with the formation of specific antibodies. The most important of these is a virulicidal antibody, and subsidiary are a precipitating or agglutinating antibody and a complement-fixing antibody.

Recently much work has been done on these bodies, and the literature has been ably reviewed by Gordon (1925), who has himself made notable contributions to the subject, laying special stress on quantitative estimation; by Sobernheim (1927), who, with his co-workers at Berne, has brought forward many important and far-reaching observations; by Winkler (1925), Schultz (1928) and others.

Within the scope of this article it is only possible to touch very briefly on some of the more salient features.

Virulicidal antibody. Sternberg (1896) was the first to discover that the serum of animals immunized to vaccine possessed the property of inactivating the virus *in vitro*. This observation has been confirmed and elaborated by many workers, who have detected the substance in the blood, not only of vaccinated and variolated animals, but in the course of human smallpox and vaccination. This antibody has been shown to be in close relation to the production of immunity; it is found accompanying the onset of immunity or shortly after; it increases in strength as immunity advances, but there is no absolute parallelism, since in vaccinated animals it frequently shows a decline and may entirely disappear whilst immunity is still present. After smallpox, however, virulicidal antibodies may persist for many years and during convalescence may show inactivating power at a dilution of from 1 to 100 or 1 to 200 (Schneider, 1923). Sato (1921) found that virulicidal capacity was enhanced after revaccination, though sometimes without visible manifestation, and this was confirmed by Fujii (1922). It would seem, therefore, that immunity is increased by a revaccination, though this be confined to an immediate reaction, a view at variance with Gins's expressed opinion that a rise in the immunity level can only follow a proliferation of the virus as evidenced by vesiculation. In some recent experimental work on the vaccinal antibody, Andrewes (1928) could find no evidence that the virus was killed by the antibody or that any stable union took place between them. Virus or antibody were respectively recoverable from serum-virus mixtures containing excess of the other.

At the beginning of this section it was pointed out that in vaccination of variolous patients after exposure and before the disease is definitely established the vaccinal response fails about the fourth or fifth day of the disease (third of the efflorescence) or even earlier. At this very early stage sufficient antibody has been evolved to inactivate completely vaccinia virus. If, however, the vaccination is performed at the end of the incubation period, it may run its full course concurrently with the focal eruption of smallpox. In this case the antibody present exhibits no inactivating effect on the vaccinia virus. The virulicidal action seems to be confined to the incipient stage, and has no effect on the virus that is already partly established and is in process of full growth. In close connection with this effect on vaccinia is the fact that the virulicidal antibody, though gaining in strength day by day, exerts no appreciable action on the smallpox virus present in skin lesions even to the very end of the disease, since scabs containing active virus may still remain attached to the body, although the patient is quite convalescent and fully immune. Antibody, it would appear, may have sufficient power to inactivate its own originating virus *in vitro* but be incapable of performing the same feat *in vivo*.

Precipitin. Recognition of a precipitating antibody as a constant factor in immune variolo-vaccine sera is mainly due to the researches of Tomarkin and Suárez (1917), who obtained their most noteworthy results with heated antigen. Previously, Tanaka (1902) had described a flocculation of vaccine lymph with variolous serum. Von Pirquet (1906), Sato (1921) and others failed to obtain confirmatory evidence, but Paschen (1913) and Winkler (1925) elicited positive results. Gordon (1925) obtained evidence of definite agglutination with immune sera and a calf lymph antigen diluted 1 in 20, with rabbit lymph antigen in dilution of 1 in 40 to 1 in 140, and also with variolous material as antigen. Burgess, Craigie and Tulloch (1929) also claim success with the flocculation test as a diagnostic agent.

Complement-fixing antibody. Numerous observers, of whom Jobling (1906) was the first, have reported positive results with the complement-fixation test. The most constant results were those produced by the serum of smallpox convalescents. Netter and Urbain (1925) found no differentiation in tests with smallpox and alastrim material and consider this as evidence of their identity. On the other hand, many workers have failed to obtain satisfactory results, including Haendel, Gildemeister and Schmidt (1921). Gordon (1925), however, adopting careful quantitative procedures, succeeded with antivaccinia serum in detecting vaccinia virus up to a dilution of 1 in 2,000, and with variolous material to 1 in 1,800, and considers that both the agglutination and complement-fixation tests may render valuable assistance in the diagnosis of smallpox. On the other hand, a note of caution has been sounded by Schultz, Bullock and Lawrence (1928), who think that some at least of the positive reactions obtained with precipitin and complement fixation may have been due to the presence of concomitant bacterial antigen. They obtained no evidence of specific complement fixation when immune sera prepared from the rabbit with pure brain virus were tested against cutaneous vaccinia or brain virus antigens. When complement fixation was produced with cutaneous vaccinia virus against brain virus antigen a similar reaction was observed in the presence of antigens prepared from bacteria cultured from the cutaneous lesions. Similarly, no evidence of specific precipitin was found.

Passive Immunity and Serum Therapy.

Raynaud (1877) showed that serum from an immune calf gave protection to another calf against subsequent vaccination. Similar experiments were made by many other observers, using as well as the calf other animals such as rabbits, apes and dogs, and it was generally recognized that the serum of animals immune to vaccinia contained substances which could set up immunity when transferred to other animals. Most of this experimental material was derived from animals which had been vaccinated in a more or less normal way and little attempt was made to hyperimmunize, so that it was necessary to employ very large doses of the immune serum in order to produce an appreciable degree of immunity, and this was especially so when curative effects were desired. With more massive and repeated infection a higher grade of immunity was reached, and Haendel, Gildemeister and Schmidt (1921) obtained an antivaccinial serum capable of neutralizing twice the volume of vaccinia virus. It was also found that protection could be transmitted *in utero* through the immunization of the mother. Further, it was shown that the serum of smallpox patients and convalescents as well as those who had been vaccinated possessed the same properties and that the transmission of such serum had both preventive and curative powers. Much of the work on this subject was due to Béclère, Chambon and Ménard (1899), Henseval and Convent (1912) and Camus (1912–13). In many of the observations, however, especially in regard to fœtal immunity, proof is lacking that the serum was entirely free from the originating virus, but in spite of this defect there can be no doubt that immune serum can be used with considerable effect for the production of immunity.

Practical application for the cure or amelioration of smallpox has not been carried out so far systematically. The best known instance is that of Teissier and Marie (1912), who, using smallpox serum in doses of 25 to 100 c.cm., treated 13 cases of smallpox with some appearance of success.

It is not unreasonable to hope that the sera of hyperimmunized animals raised to a high titre may prove a valuable agent for the cure and alleviation of severe smallpox. At the same time means will probably need to be taken to circumvent the streptococcus which is so often a disastrous factor in smallpox, by associating the antivariolous serum with an autogenous antistreptococcic serum.

Production and Preparation of Vaccine Lymph.

Procedures at the Government Lymph Establishment of the Ministry of Health.

Vaccinifer. Calves are used, mostly of English shorthorn breed, 4 to 6 months old, weighing approximately 260 lb. on the average. Males and females are used indiscriminately, males generally having the better skin. The calves as far as possible should have unpigmented skins; those with red and white coats give, as a rule, the best results.

Housing. The stables are in two sections—quarantine and vaccination, each comprising two stables of 20 stalls each. The two sections are well apart from one another. The stables are spacious and well ventilated, with walls and floors so arranged that they can be hosed down and kept scrupulously clean. The stalls in each stable are arranged ten a side, and are made of galvanized iron bars fitted toward the head end with movable gates, which, when closed, effectually prevent the animal from licking the vaccinated surface, but in no other way interfere with its freedom and comfort. Between the head of the stalls and the side walls are gangways allowing free air-space to the head of the animal. Each stall is floored with a movable teak batten on which the litter rests. The battens are thoroughly cleaned each day. The stables are used alternately week by week. Calves are received into the quarantine stables and kept under observation for a week, during which they are carefully inspected, groomed and their daily temperatures taken. At the end of the week, if their condition is satisfactory, they are transferred to the vaccination stables, where their condition is similarly investigated. The day before vaccination they are taken to the operating room, placed on tables, and the area to be vaccinated carefully shaved. The next day the area is lightly shaved again, washed thoroughly with soap and warm water, rinsed with sterile water, and finally dried with sterilized gauze sponges. No antiseptics are used.

Operating room. This room is well lighted and is kept scrupulously clean; it contains twelve operating tables, made of oak, and so constructed that the animal can be kept clean and maintained in position with as little inconvenience as possible.

Vaccination. The area to be vaccinated comprises one side of the abdomen from the inner side of the thighs to as far forward as the ribs. Vaccination is by linear incisions, each 3 to 6 inches long and about ½ inch apart. The incisions are made very lightly with a sharp scalpel so as not to draw blood and the seed lymph is rubbed into the incisions with a metal spatula. All instruments and appliances are sterilized. After vaccination the calf is returned to the vaccination stable and freshly bedded down daily or more frequently if necessary. The temperature is taken twice daily. No covering is placed over the vaccinated area.

Condition of calf during the vaccination process. The calf shows no disturbance during this period. Neither appetite nor general condition suffer. On the third day the temperature, which is normally about 102° F., rises, reaching a maximum of 104 to 105° on the evening of the fourth day, but it begins to fall on the morning of the fifth day and becomes normal in a day or two.

Seed lymph. The seed lymph used is derived from a strain of calf lymph received from Cologne in 1907. Its quality has been maintained by passage from calf to calf and by cutaneous passage through the rabbit. For calf passage it is necessary to select the best vesicles from a vaccinated calf which has given an excellent yield, and to transfer these to another suitable animal and so on, taking special precautions that the vesicles transferred shall always be of the highest quality. In this way lymph can be carried on from calf to calf for several generations and the quality maintained, but this is more readily and more certainly effected by the use of rabbits. Young rabbits, six to eight weeks old, weighing about one pound each, and with supple unpigmented skins, are employed. They are carefully shaved on one side, the shaved area is then abraded lightly but sufficiently to leave it pink all over, and the vaccine lymph derived from another rabbit or calf is rubbed in gently with a sterile spatula. Generally a confluent vesicular eruption results in 48 hours, but this may be delayed to 78 hours. If the eruption is not satisfactory at that period it should not be taken. The animal is then killed, the skin stretched, and the eruption collected with a Volkmann spoon. Generally six rabbits are inoculated at one time and the product pooled. The collected material, averaging about 0·25 gm. per rabbit, is ground up finely in a mortar and mixed with six times its weight of 50 per cent. sterile glycerin and distilled water which has been titrated and brought to alkalinity. The resulting emulsion is placed in cold storage at −11° C. till required for use, bacteriological examinations being made from time to time. Generally seed lymph is a year or more old before its employment for the vaccination of calves, at which time it is further diluted with 50 per cent. glycerin and water to as much as 1 in 50 according to consistency. Calf lymph when used as a seed lymph is diluted to a similar extent.

Collection. On the fifth day, or 120 hours after vaccination, the vaccinated surface on the calf is well washed with soap and water, rinsed with sterilized water, and dried with sterilized sponges. The inoculated incisions at this time should show regular development of vesicles and these are collected by means of a Volkmann spoon line by line and transferred to sterile bottles. This constitutes the pulp.

Slaughter of the calf. After the collection of the pulp the calves are removed to a slaughterhouse where they are slaughtered in the presence of a veterinary surgeon employed by the Ministry of Health. He makes an autopsy on each animal, which includes an examination of the mediastinal and mesenteric glands as well as the visceral organs, and reports thereon. Unless the calf is certified as perfectly healthy the lymph collected from it is not issued.

Preparation. The pulp from each calf is treated separately: its weight is ascertained and four times this weight of diluent is gradually added during the trituration process. The diluent consists of 50 per cent. sterilized glycerin and distilled water titrated, alkalinized with sodium carbonate and brought to a pH of 7·6, with 0·1 per cent. oil of cloves added. The pulp is first examined macroscopically and any hairs or foreign bodies are removed, it is then passed through the triturating machines three times with the gradual addition of the diluent and the resultant emulsion is tested to see that it has been brought to a sufficiently fine state of division and homogeneity by dispersing a loopful in distilled water, when no distinct particles should be visible to the naked eye. Each batch of lymph is filled into tubes of suitable size, which are carefully corked and sealed and then placed in the cold store at −11° C.

Triturating machines. Machines modified from the Chalybaus machine are used. These consist of a vertical spindle carrying a diminishing thread which revolves on ball-bearings in a split cylinder, the two halves of which are accurately clamped together. The machines are actuated by electric motors.

Bacteriological examination. Aerobic and anaerobic cultures are made from the lymph at this stage, the first on nutrient agar, and the second in glucose agar under anaerobic conditions and in meat media in Smith's tubes. The examinations are repeated periodically till the lymph is issued. Lymph in which anaerobic organisms or streptococci are detected is not issued. The bacterial content at glycerination averages about 4,000 organisms per mgm., comprising *Staphylococcus aureus* and *albus*, *aureus* as a rule predominating, and various common saprophytes, *B. mesentericus*, moulds, sarcinæ and yeasts.

Owing to the action of glycerin combined with oil of cloves extraneous organisms are gradually eliminated, the greatest fall taking place in the first and second weeks. The fall can be hastened, if desired, by exposure of the lymph to a higher temperature, 15° C., which exposure, however, should not be prolonged for more than a week at a time. When the organisms are eliminated or reduced to five or less per mgm. the lymph is ready for issue.

A large stock of lymph is held in reserve, stored continuously at −11° C., and is issued in rotation; the usual age at issue is, approximately, two years from the date of collection.

Quality of the lymph. This is estimated primarily by the appearance of the vesicular eruption on the calf; the development, shape, size, regularity and continuity of the vesicles along the lines of incision being the chief criteria. A well-developed lymph shows a regular and continuous vesicular band along each line inoculated, having an elevation of $\frac{1}{12}$ to $\frac{1}{8}$ inch and a diameter of $\frac{1}{8}$ to $\frac{1}{4}$ inch. The edges of the band where it springs from the normal skin are parallel, there are no indentations and no signs of individual vesicles. In colour the band is pearly white tinged with pink; umbilication is seen as a shallow depression running centrally and evenly along the band and of a slightly darker pink colour. At the ends of the incisions the band is rounded off bluntly and shows no tapering. To the touch the vesicular band is firm but elastic. There is no areola or only a faint pink blush on the skin at the edge of the band. Experience shows that observations on these lines allow the potency and duration of potency of vaccine vesicles to be estimated, whilst still on the calf, with great accuracy.

Laboratory tests. The Therapeutic Substances Act laid it down that each batch of lymph should, before issue, be tested on animals in a thousandfold dilution. Four tests were suggested for this purpose and each was found to give a satisfactory estimate within the limits prescribed. It was agreed that the lymph before application should be finely ground up so that filtration was unnecessary and that the lymph as diluted for distribution may be considered potent if, when further diluted a thousandfold, it will produce the following results:

1. *By Gins's method;* 72 hours after the guinea-pig's cornea is scarified and inoculated with such dilution, a well-marked opacity of the entire cornea (keratitis vaccinica) should be apparent to the naked eye.

2. *By Sobernheim's method;* the dilution inoculated on to the skin of a rabbit in three linear incisions, should, once the trauma has passed away, begin to produce a specific reaction in three days. This reaction consists of redness and papilliform infiltration at isolated points along the lines of incision. Papules (of the size of a pea) and in some cases pustules are formed. Sometimes papules and pustules appear between the lines of incision. The specific eruption shows crusts and scabs. With a more virulent vaccine lymph the papular infiltration spreads along the lines of incision. In addition, the inoculation of the scarified rabbit's cornea on the same animal with dilutions of 1 in 5,000 and 1 in 10,000 should show a parallel reaction (keratitis) with that on the skin.

3. *By Groth's method;* 72 hours after intradermal injection into the depilated or shaved skin of the back of a rabbit 0·1 c.cm. of vaccine lymph diluted 1 in 1,000 should produce an area of local infiltration and redness which can be clearly seen and recognized by palpation. The reaction follows in 24 hours with the strongest dilutions, but is delayed with the weakest until the fourth or fifth day. The infiltrated areas can be compared and measured by calipers.

4. *By the Calmette-Guérin method;* 1 c.cm. of vaccine lymph diluted to 1 in 1,000, when distributed over the prepared area of the rabbit's skin immediately after it has been shaved, washed and dried, should

produce an eruption of isolated vesicles numbering from 3 to 4 per sq. cm. of the field inoculated. The results should be recorded on the fifth day after inoculation.

All these methods are suitable for the estimation of potency, but the one employed here and advocated is a modification of the Calmette-Guérin method. This consists in shaving the back and sides of a full-grown rabbit, with a non-pigmented skin, dividing the shaved area, after washing and drying, into four equal parts, which are severally carefully but lightly abraded over the whole area until the skin becomes pink. To each area is applied 0·2 c.cm. of a 1 in 1,000 dilution of each of four different batches of lymph, so that in a lymph diluted four times originally the actual amount of vaccine pulp applied to each area is 1/25,000 gm. The amount inoculated, 0·2 c.cm., is accurately measured and blown out into a watch-glass, from which it is transferred to the prepared area by a bone spatula, so that it completely covers the area. The result is a confluent eruption of vesicles on each area, which, the fifth day after the inoculation, is inspected and reported on.

It is essential that the skin should be deliberately injured and not left to the chance traumata of a razor.

If the result is not satisfactory the cause is invariably insusceptibility of the rabbit. The experiment is repeated on a more suitable animal, and, given this condition, in the writer's experience no batch of lymph has failed to give a confluent eruption.

In addition to the animal tests it is customary to make a small first issue for the vaccination of children which serves to indicate the quality of the lymph.

Procedure in other Vaccine Institutes.

Information as to this was obtained through a questionnaire drawn up by the Smallpox and Vaccine Commission of the League of Nations, and issued to various vaccine institutes. The answers were summarized by Groth (1929).

Vaccination of the Human Subject.

It is recognized in all civilized countries that vaccination and revaccination at suitable intervals afford a sure protection against smallpox, and that by no other means at present known can this be obtained.

Methods of Vaccinating.

The procedure advocated by the Committee on Vaccination (1928[2]) and embodied in the recent Vaccination Order (August, 1929) is to vaccinate in a single linear incision or scratch not more than ¼ inch long, merely through the epidermis. This is in place of the four insertions previously enjoined, but multiple insertions up to this number are not precluded in cases in which the vaccinee or public vaccinator desires to obtain additional protection at one operation, as, for example, in the face of virulent smallpox or where exposure to infection is probable.

Other methods of vaccinating are the multiple puncture or pressure method through a drop of vaccine (White, 1925; Leake, 1927); the drill method, in which a chisel having an edge 2 mm. wide is used, one revolution removing the superficial layer of epidermis (Force and Stevens, 1918); a method advocated by the Union of South Africa (1924) and others, of making two or three parallel incisions $\frac{1}{4}$ inch long and $\frac{1}{16}$ inch apart through a drop of lymph; such insertions may be single or multiple. Cross-hatching is severely deprecated and multiple insertions should be not less than one inch apart. Vaccination may be through a drop of lymph or the lymph may be applied to the trauma either simply or gently rubbed in. Some evidence has been given (Ministry of Health Report of Committee on Vaccination, 1928[3]) that the former gives less local reaction than the latter.

In all cases the procedure should be treated as a surgical operation, and rigorous precautions taken to exclude any possibility of septic contamination.

The one mark vaccination has been advocated because it is hoped by these means to lessen the local reaction, to mitigate or obviate complications, and to render the operation as little discomposing to the vaccinee as possible. To the same end trials with dilutions of lymph beyond that usually employed are being made and the results so far obtained suggest that a dilution of 1 in 100, though possible in skilful hands, is liable to failure, that 1 in 50 gives good results but requires an accomplished technique, and that 1 in 10 may be looked upon as quite reliable. The degree and duration of immunity produced by one mark vaccination, or by such dilution, has, however, so far not been determined. The Committee on Vaccination (1928[2]), however, recommended that the primary vaccination as carried out on above lines should be followed by at least two further similar vaccinations, viz. on entering and leaving school.

Protection of the vaccinated surface. Advocates of the pressure method state that one of its advantages is that no covering or protection is required except perhaps a loose sleeve, and this treatment is probably the best. There is little doubt, however, that in a workaday world, after ordinary cutaneous vaccination, protection from dirt and from injury to the maturing vesicle is necessary. So far, the best treatment appears to be a pad of aseptic gauze, wool or lint, secured to the arm by tape or bandage and not by strapping. Celluloid covers and similar appliances should be avoided.

Course and Symptoms of Vaccination.

Primary vaccination. There is an incubation stage of about three days during which little change occurs at the inoculated site except slight redness and swelling. During this period the temperature falls slightly below normal. Papulation can be distinguished on the third or fourth days, the papules becoming vesicular on the fourth or fifth days. With the development the temperature rises, gradually reaching its maximum, which varies from 100 to 104° F. on the ninth day. As the vesicle forms, around it appears an area of redness, the areola, which extends some distance from the vesicle, attaining its largest dimensions on or about the eleventh day. The vesicles also increase in size and reach their maxima on the twelfth to thirteenth day, after the areola has begun to subside and when the temperature is definitely falling and is nearly normal. The extent of the areola, the degree of vesiculation and the temperature, bear no constant relation one to the other.

The vesicles begin to dry up at about the eleventh day and complete the process by about the fourteenth, the scabs falling off normally about the twenty-first day.

Slight febrile disturbance may be noted as vesiculation proceeds, frequently with headache and malaise on the seventh or eighth day, and some enlargement of the axillary glands. From the fourth day onwards in a number of cases the spleen becomes palpable, with a maximum enlargement about the eighth day. Whether the enlargement is specific and due to the activities of the vaccine virus or to the febrile condition and associated hæmatopoietic modifications, is yet to be determined (Acland, 1899; Cory, 1898; and others; Commission on Vaccination, 1928[4]).

Sobotka (1893), von Pirquet (1907) and others have described a leucocytosis in the first few days of vaccinia; this was not found in the investigation made for the Committee on Vaccination, but there was noted a diminution of the polymorphonuclear leucocytes, and a rise in the lymphocytes to about 48 per cent., the ratio of the two varying inversely with the temperature. A similar condition has been described by Hoffmann (1923), Pantasis (1924) and others in smallpox.

Scarring. With the fall of the scab a scar is left, which, at first dusky red, becomes in time white and foveated. The foveation depends largely on the depth and intensity of the vesicular process.

Revaccination. The degree of reaction in a revaccination depends on the amount of immunity remaining after the primary vaccination. The less the immunity the closer the revaccination approximates to a normal primary vaccination, and the higher the immunity the more accelerated and the more diminished the local reaction. It is convenient to divide revaccinations into three classes: (1) vaccinias, those in which there is practically no residual immunity, and the reaction is parallel with a normal primary vaccination; (2) vaccinoids, in which there is a fair degree of residual immunity and the reaction proceeds as far as vesiculation, which dries up about the sixth day and then rapidly scabs off; (3) the immediate or immunity reaction in which there is a high degree of immunity and the reaction never proceeds as far as vesiculation, but only slight swelling with or without papulation, which fades away leaving a brownish stain. The immediate reaction should always be controlled by an uninoculated parallel incision.

Complications.

1. *Those specific to vaccinia.* (*a*) Auto-infection, in which the vaccinia becomes more or less widely distributed over the skin by mechanical transference from the inoculated site. This generally takes place about the end of the first week and is an indication that antibodies are insufficiently generated. Serious results are not usual, but may result if a sensitive organ, such as the eye, is affected.

(*b*) Generalized vaccinia, in which the vaccinia is distributed throughout the body, manifesting itself visibly on the skin and perhaps mucous membranes of the mouth, &c., by natural extension from the inoculated site. This again is, no doubt, due to inadequate and tardy formation of antibody and has already been alluded to (p. 98).

2. *Those not necessarily specific to vaccinia.* Certain rashes are occasionally met with occurring commonly from the third day onwards, but sometimes not until the tenth day. Similar appearances are seen in other febrile conditions, and appear to be due to chemical irritation rather than to virus infection. These rashes are of three kinds :

(*a*) An urticarial or morbilliform rash generally most marked on the vaccinated arm but extending also to the trunk, and frequently very irritating ; this rash is similar to that seen after injection of antitoxic sera.

(*b*) An erythematous rash not uncommonly displaying petechial hæmorrhages, found mainly on the vaccinated arm but sometimes extending to the trunk.

(*c*) A papulo-vesicular rash in which some papules become vesicles, but many dry up and scab off in the papular stage. This occurs on the vaccinated arm or on the face, back, &c. It is not clear whether this rash is an abortive attempt at generalization or merely the result of irritation. In such a rash active virus capable of transference to animals has not been obtained from the vesicles, but bodies similar to Guarnieri's corpuscles have been found (Gans, 1929). In addition, petechial hæmorrhages occur around the vaccinated site, especially where the local reaction is intense. It is possible that this and the erythematous type are specific to vaccinia, for they have their analogues in smallpox.

3. *Complications following infection of the wound.* (*a*) Owing to the discontinuance of arm to arm vaccination and the use of animal lymph, infection with syphilis has been entirely obviated.

(*b*) The most dreaded complications of vaccination were erysipelas and septic infections, including vaccinal ulceration and glandular abscess. These, by rigorous attention to asepsis and by care in the preparation of, and use of, animal lymph, have been largely and can be entirely prevented.

(*c*) Tetanus in this country is of extreme rarity and is only possible where there has been gross contamination of the wound.

Complications arising from affections of the central nervous system, such as post-vaccinal encephalitis, are dealt with in Chapter VIII.

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CHAPTER VIII. ACUTE DISSEMINATED ENCEPHALOMYELITIS (WESTPHAL) AS AN INDEPENDENT DISEASE AND AS A SEQUEL OR COMPLICATION OF CERTAIN VIRUS INFECTIONS (VACCINIA, VARIOLA, MEASLES, RABIES, &c.).

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THE disease of the nervous system to which Westphal (1872) gave the name of 'acute disseminated encephalomyelitis' has recently assumed importance, owing chiefly to an increase in its incidence as a complication of vaccination. Clinical signs of encephalitis or paraplegia have come on within a fortnight of vaccination in a certain proportion of cases both in England and Holland. Apart from vaccination, a disease exactly similar both in its clinical manifestations and in the lesions found on histological study of the nervous system has occurred, sporadically it is true, but with increased frequency of late years, after other infective diseases, especially smallpox and measles, and cases have also been seen which bore no relation to any exanthematous fever. The recognition of this fact has made it necessary to reconsider a view which some few observers have held, that the symptoms are due to direct attack on the nervous system by the virus of vaccinia, and to attempt the formulation of some ætiological theory which, while assuming that the disease constitutes a clinico-pathological entity, would at the same time allow for its relationship to the exanthemata.

Characteristics of the Disease.

Encephalitic and myelitic forms of the disease are very often combined. When this is so the cerebral symptoms are usually so severe that the symptoms of paraplegia are likely to be obscured. In other cases lesions are found *post mortem* throughout the nervous system in cases which have presented only paraplegic symptoms during life. There are, however, cases in which both clinically and pathologically the symptoms are confined either to the brain or to the spinal cord. In the encephalitic form of the disease the chief symptom is somnolence often associated with epileptiform fits or myoclonic twitchings, frequently deepening into coma and often ending fatally. There may be paralyses of cranial nerves, hemiplegia and, more rarely, transient amaurosis followed by temporary achromatopsia. Trismus is frequently noted and may lead to the diagnosis of tetanus. In many cases, especially in children, the symptoms resemble closely those of meningitis, and this diagnosis is frequently made. Recovery is usually fairly rapid and complete. The patient often passes through a phase which resembles the stage of recovery from concussion and which may last for days or even for weeks in some cases. Considering the severity of the initial symptoms, sequelæ are usually slight, but some degree of mental enfeeblement or epilepsy, especially of the *petit mal* type, sometimes follows the attack. Sequelæ suggesting focal damage to the brain, such as ataxia or hemiparesis, are less commonly observed.

In the paraplegic form the disease usually begins with retention of urine followed by progressive paraplegia, of more or less acute onset or of an ascending type, with some degree of sensory loss. The paralysis, which is at first flaccid, assumes the characters of a spastic paraplegia after a few days, and very rarely is there any local muscular wasting. As in the cerebral form of the disease, recovery is usually remarkably complete, the disturbance of the sphincters of the bladder and rectum often being the last symptom to disappear.

Pathological Characters.

Histologically the disease is characterized by perivascular zones of demyelination, accurately centred on a vessel and following it throughout its course. These zones vary in width but are usually narrower in radius than the diameter of the vessel. In the brain they are found most commonly in the subcortical white matter, especially in the white centres of the convolutions, and in the thalamus, midbrain and pons. In the cord the vessels which run outwards from the anterior horns through the antero-lateral columns are usually surrounded by demyelinated zones. The dorsal columns may be affected in some cases but often escape more or less completely, a fact which accounts for the dissociated nature of the sensory loss which is sometimes observed.

Not infrequently zones of demyelination are found around the margin of the cord, or on the lips of the ventral median fissure, and these are quite independent of the arrangement of vessels. Similar zones may surround the ependyma of the lateral ventricles, and seem to be often associated with the marginal zones in the cord.

The cellular structure of the demyelinated areas depends partly on the severity of the disease and partly on its duration. In cases which terminate within two or three days of the onset, the chief evidence of demyelination is an increased number of cells in the perivascular zones, but even at this early stage some destruction of myelin can be seen by appropriate staining methods. The cells constituting this increase are chiefly microglial in origin. Lymphocytes and polymorphonuclears may also be seen, but are scarce. At a rather later stage, toward the end of the first week, rounded microglial cells containing fine fatty granules are abundant, and some swelling and possibly proliferation of the neuroglial cells are also seen. Later still the demyelinated zone is cleared of lipoid ; a few fat-granule cells may still be found in it, but the majority have migrated to the walls of the vessels where they fill the adventitial (Virchow-Robin) spaces.

In addition to this characteristic demyelination of perivascular zones there is usually some degree of infiltration of the adventitial lymphatics with cells of the lymphocyte class. This infiltration affects not only the vessels on which the zones of demyelination are centred, but many other vessels both in the neighbourhood and sometimes also apparently at a distance from such zones. In some cases the infiltrating cells are chiefly lymphocytes; in others, lymphoblasts and plasma cells are fairly numerous, but these are distinctions of degree rather than of kind. The amount of infiltration varies greatly from case to case; in some it is very slight, forming isolated rings never more than one cell thick; in others it is intense, and almost every vessel in the affected areas is surrounded by a thick cuff. There appears to be little or no relationship between the severity of the disease and the degree of this adventitial infiltration; it appears early, but is usually slight within the first two days, and becomes most intense toward the end of the first week, thereafter disappearing fairly rapidly. But it may be absent or very slight at all stages.

Vascular congestion, sometimes of sufficient degree to lead to petechial hæmorrhages, is usually present in the earlier stages, and has been intense enough in some cases to obscure the more characteristic lesions.

The disease has a tendency to come on at a definite time after each of the exanthemata which it follows. Thus in the great majority of cases it has followed vaccination with an interval of between 10 and 14 days. The records with regard to smallpox are less abundant, but as a general rule the onset of drowsiness, fits or paralysis is usually delayed until the stage of vesiculation or pustulation. In the case of measles, recent instances have been sufficiently numerous to establish the fact that the nervous disease almost always comes on after the rash is out and when the fever has subsided, usually at the end of the first week of illness, but sometimes it may be delayed till a fortnight or three weeks after the onset. Similar symptoms with, in fatal cases, an apparently similar pathological picture have been known to come on during antirabic treatment, usually at least seven days after treatment has begun.

Nervous symptoms of a similar nature have been described as coming on after such fevers as typhoid (Ebstein, 1872; Landouzy, 1880); varicella (Wilson and Ford, 1927; Winnicott and Gibbs, 1926); mumps (Bedingfield, 1927); scarlatina (Grinker and Stone, 1928); but there is no satisfactory evidence that the pathological picture is the same in these cases. The writer has, however, examined the nervous systems of two cases in which acute paraplegia followed a febrile illness diagnosed as influenza. One patient died after a few days and the other after a few weeks. In the cords of both cases lesions were found which were identical with those seen at similar intervals after the onset of post-vaccinial encephalomyelitis. The evidence regarding the encephalomyelitis which may follow measles is also now sufficient to establish its clinical and pathological identity with that which follows vaccination and smallpox (Barlow, 1886; Wohlwill, 1928; Greenfield, 1929).

The latter disease should not, therefore, be regarded as a separate entity, but should rather be grouped along with the forms which may follow other febrile illnesses. For this disease it is proposed to retain the name 'acute disseminated encephalomyelitis', which Westphal used to describe the pathological picture of two post-variolar cases. This name is satisfactory in so far as it indicates the distribution and the essentially inflammatory nature of the pathological lesion. Being based on morbid anatomy it does not postulate any special ætiology, nor suggest any definite clinical picture, and is only applicable to those non-fatal cases in which the clinical picture leaves no doubt as to the diagnosis. In the absence of fuller pathological data we are not justified in applying it to all cases of acute encephalomyelitis following eruptive or other febrile diseases.

The question has been raised in this connection whether similarities in clinical course and in pathological lesions are sufficient for the separation of a condition as a disease entity, when the ætiological or at least the exciting factor appears to be diverse. It has thus been suggested that the virus of many different fevers may occasionally, by alteration of virulence, produce changes in the nervous system which differ only in intensity. To this question an answer has been sought by experiment, but so far without success, as the characteristic histological lesions of acute disseminated encephalomyelitis have never been reproduced in experimental animals. Most of the experiments have been directed towards deciding the part played by vaccination in causing the disease. These experiments are very numerous and varied and only a short summary of those which seem most important can be given here:

(1) Bastiaanse, Bijl and Terburgh (1926), Levaditi, Lépine and Troisier (1928), Kraus (1927), Perdrau (1928^2) and Maitland (1928) tested the brain of fatal cases for vaccine or other virus, with negative results. McIntosh and Blaxall (1928), by passing immature crusts from rabbit to rabbit, obtained typical vaccine vesicles at the third passage. Maitland tested two of these brains by the same technique, but failed to find vaccine virus.

(2) Levaditi and Bijl (see Bastiaanse, Bijl and Terburgh, 1926) (in one rabbit out of many) produced encephalitis in rabbits with the cerebrospinal fluid of cases of post-vaccinial encephalitis. They were able to transmit the encephalitis in series, but were unable to determine the nature of the virus, which was neither vaccine nor herpes virus.

(3) Vaccine virus has been recovered from the brain of cutaneously vaccinated rabbits by Lucksch (1927), Levaditi and Nicolau (1925), Blanc (1926) and Blaxall (1928). Blaxall has also recovered it from the brains of calves and a monkey.

(4) Intracerebral inoculation of vaccine virus produces a fatal meningo-encephalitis in rabbits. Corneal and cutaneous vaccination is practically never followed by encephalitis.

(5) Numerous experiments have also been made to determine whether vaccination raised the virulence of other viruses (especially herpes encephalitis), with inconclusive results. These experiments have only an indirect bearing on the subject.

Experimental work on the encephalomyelitis following antirabic treatment has given similarly inconclusive results. The pathological picture of rabies in man, dog or rabbit lacks the characteristic features of acute disseminated encephalomyelitis. Babes and Mironesco (1908) inoculated the brain of their fatal case into rabbits with negative results.

The evidence, therefore, so far as it goes, favours the view that ' acute disseminated encephalomyelitis ' is a disease *sui generis*, which is only indirectly induced by the virus of the antecedent disease. The very definite relationship which exists between the exanthem and the onset of nervous symptoms may be accounted for in various ways. It might be supposed in the first place that the virus was in some way associated with the virus of the infection, so that the patient became infected with both types of virus at the same time. Thus, Perdrau (1928^2) has shown that it is possible for two viruses, such as those of vaccinia and herpes, to be transmitted together in series through several rabbits, and to be separated by the inoculation of rabbits previously immunized to one or other virus. To this theory there are, however, many objections. In the first place the cases following measles or smallpox are almost always isolated, and there is no evidence of case to case infection with the nervous disease. In the post-vaccinial cases, the disease has followed vaccination by all makes of lymph, and in some instances when two members of a family have been vaccinated at the same time with different kinds of lymph, both have developed nervous symptoms. The fact which is now generally accepted that the disease has been more common during certain years suggests a certain epidemicity, and is most easily explained on the theory that the presumed virus of encephalomyelitis spreads widely among the community, but does not produce any symptoms unless the patient, while carrying the virus, is infected with an exanthem or has his resistance to the virus lowered in some other way. There are two chief ways in which this might take place :

(1) The general bodily resistance of the patient might be lowered by the febrile illness, or the virus of encephalomyelitis might have its passage to the nervous system facilitated by the febrile illness. It is known that after the injection of diphtheria or tetanus toxin or of tuberculin (Stern, 1923), the barrier between the blood-vessels and nervous tissue is weakened, and it is conceivable that the specific fever might have a similar effect.

(2) The virus might be altered by the exanthem in such a way that its virulence for nervous tissue was raised. This view is favoured by the special relationship of variola and measles to the nervous disease, and by the experiments of Levaditi and Nicolau (1925) and of Zurukzoglu (1927) which indicate that the neurotropic virulence of either herpes or vaccine virus may be increased by the previous or simultaneous inoculation of the other virus. These experiments, however, were not confirmed by the later work of Perdrau. On the whole, the evidence favours the theory

that the exanthem in some way alters the bodily resistance of the patient rather than that it produces any direct effect on the presumed encephalomyelitic virus.

Pathogenesis.

The virus in disseminated encephalomyelitis has a peculiar power of causing rapid demyelination. It has been seen that this occurs in three situations: (1) round blood-vessels, (2) round the margin of the cord, and (3) round the walls of the ventricles. These three situations are all in relation to the cerebrospinal fluid, and it is, therefore, possible that the virus is spread by this medium. The rarity of lesions in the lenticular nucleus, at least in the globus pallidus, and their frequency in the subcortical white matter and thalamus, which are nearer to the mouths by which the perivascular channels (Virchow-Robin spaces) communicate with the cerebrospinal fluid, also favour this view. A spread directly by the blood-stream has been suggested by Perdrau (1928[1]), but is rendered unlikely by the patchy nature of the lesions in some cases, and by their situation on the margin of the cord and in the walls of the ventricles in others. It is evident that the infection is rapidly overcome in the majority of cases. Unless death occurs within a week or ten days it is usually not directly due to a spread of the disease, but to septic complications in the chest or urinary tract. Relapses are very rare, but have been described even at fairly long intervals.

It has been suggested by several writers that other forms of demyelinating disease of the nervous system are caused by the same or a closely related virus. Of these diseases the commonest is disseminated sclerosis, and it was strongly held by Pierre Marie (1895) that this disease frequently followed smallpox, typhoid and other specific fevers. Recent experience does not, however, support this view. Nor is there much evidence that cases which begin as acute disseminated encephalomyelitis are likely to develop disseminated sclerosis later. Two other much less common forms of disease should also be mentioned, Schilder's encephalitis periaxialis diffusa, and ' neuroptico- ' or ' diffuse myelitis '. With regard to the first of these diseases no relationship has been proved either to a specific fever or to a previous attack of disseminated encephalomyelitis, but with regard to the second we are on less certain ground, as no very sharp pathological distinction can be drawn between the diffuse and disseminated forms of myelitis.

So long as it remains impossible to reproduce any of these forms of disease at will in animals, and so to study them experimentally, we are likely to remain in complete ignorance of the nature of the viruses which cause them, and any hypotheses as to their relationship to one another must be accepted with caution.

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CHAPTER IX. ANIMAL POCK DISEASES.

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Introduction.

MANY of the domesticated animals are subject to an eruptive skin affection of a pock-like nature and hence the term animal pock diseases. These disorders occur only in animals which are aggregated in numbers, as sheep, cattle, goats, swine, horses and poultry. There are no authentic records of the occurrence of the spontaneous natural disease in the dog, cat, rabbit, monkey, camel and buffalo, all of which are susceptible to inoculation. Monkeys, as mentioned in Chapter VII, have been observed to contract variola from man (Bleyer, 1922). There are thus two classes of animals subject to the disease—mammals (including man) and birds. The disease in mammals Bollinger (1877) differentiated into two groups, firstly, that in which it is of an epidemic nature and freely infectious between animals of the particular species, comprising human variola and sheep-pox ; secondly, that in which it is always of sporadic occurrence and communicable only by contagion, comprising goat-pox, swine-pox, horse-pox and cow-pox.

Bidault (1927) suggests as a tentative grouping :

(i) Resembling human variola : sheep-pox, goat-pox, swine-pox, bird-pox. Bidault thinks that to this category might perhaps be added—at some distance—distemper of dogs.

(ii) Resembling vaccinia : cow-pox, horse-pox, stomatitis pustulosa of the horse and ecthyma contagieux of sheep and goats.

The writer proposes another grouping based on infectivity and whether or not the exanthem affects mucous membranes :

(i) *Epidemic.* Spread by infection between animals of the same species, as well as by contagion, characterized by fever, constitutional disturbance and a papulo-vesicular exanthem involving the general integument and affecting the mucous membranes, especially of the upper respiratory tract : human variola, sheep-pox, goat-pox and swine-pox.

(ii) *Non-infectious.* Spread only by contagion, causing less constitutional symptoms, but exhibiting an exanthem similar to above, involving both skin and mucous membranes : horse-pox and fowl-pox. To this group would be added, if further study warrants its inclusion, ecthyma of sheep.

(iii) *Non-infectious.* Spread only by contagion, exanthem of similar character to groups (i) and (ii), but almost invariably localized and not involving mucous membranes : cow-pox.

Sheep-Pox.

(Ovinia ; Variola ovina ; Clavelée ; Schafpocken.)

Description. An infectious epidemic disease affecting sheep, characterized by fever, marked constitutional disturbance, and a papulo-vesicular eruption affecting the skin and involving mucous membranes of respiratory tract and genitalia.

Symptoms. There is an incubation period of 8 to 10 days (Immermann, 1902) ; 4 to 7 (Friedberger and Fröhner, 1908) ; 4 to 8, sometimes less (2 to 4), seldom 10 to 12 (Zwick, 1927) ; an initial stage accompanied by fever, general malaise and perhaps discharge from the eyes and nose, and sometimes the appearance of a roseolar rash similar to the initial rash of smallpox. In a day or two a papular eruption appears, especially on the parts least covered with wool. Sometimes the eruption is general over the whole surface of the body, invading also the mucous membranes of the nose, mouth, pharynx and genitalia. About the fifth day the papules are surrounded with a red areola and become vesicular. On the sixth or seventh day the vesicular contents become turbid and pustular. The eruption may be discrete or sufficiently profuse to be confluent. Secondary fever follows, accompanied by inflammation of the conjunctivæ and mucous membranes of the air passages, which discharge a mucopurulent secretion. Eventually the pocks dry up and separate, leaving a depressed cicatrix. On the other hand, the pocks may not become vesicular but remain as hard nodules under the skin, often the size of nuts (variola ovina compressa). Not only may the mortality be high—10 to 50 per cent.—but not infrequently permanent damage is done to the fleece. The resemblance to human smallpox is very marked and similar variations in the type of disease are noted as V.O. hæmorrhagica, V.O. confluens, &c.

Pathological anatomy. The pocks vary much in size—from a hemp seed to a bean. Histologically the appearances are similar to those in smallpox: no fundamental differences appear to have been noted. In V.O. compressa the nodules on section show a homogeneous section, composed chiefly of polynuclear leucocytes, of which many have broken down nuclei, lodged between fragments of connective tissue. In the skin of sheep inoculated with true sheep-pox, Gins (1927) states that the tissues display the same histological appearances as in variola and vaccinia, and in the epithelial cells Guarnieri bodies can be found.

Transmissibility. Sheep are readily susceptible to vaccinia and are used for the production of vaccine lymph for human vaccination. Marchelli (1802) appears to have been the first to use sheep-pox for this purpose. He was successful and is quoted by Sacco (1809). Sacco amplified his experiments, vaccinating children directly from sheep-pox and proving them immune by subsequent variolation. He also attempted to immunize sheep by inoculating them with sheep-pox material, but found that this set up a general eruption and tended to spread the disease. Later he discovered that sheep-pox after passage through man or the calf had lost its virulence for the sheep and merely set up a local reaction at the point inoculated. No general eruption followed, nor marked constitutional symptoms, and the disease was not infectious to other sheep. He therefore inoculated sheep with vaccine (the source of the vaccine is not stated, but probably it was humanized lymph) and found that they were thereby protected against sheep-pox. Sacco's work is important ; by his careful experiments with sheep-pox and also with horse-pox he contributed largely to a wider knowledge of the animal poxes, and laid the foundation for a closer study of their immunological relations. His work, however, was ignored or contemned.

Nocard and Leclainche (1903) asserted that sheep-pox was a disease peculiar to sheep and that inoculation was without result on man, horse, cattle, goat, pig, dog, monkey, rabbit, guinea-pig and birds. Borrel (1903) found no immunity between sheep-pox and vaccinia. More recently, Bridré and Donatien (1921), working in Algiers with Algerian sheep, came to the same conclusion and consider the two diseases quite distinct. They found that vaccinia did not immunize sheep against sheep-pox, and more important still that sheep-pox did not immunize against vaccinia. Algerian sheep, it may be noted, are considered to be less susceptible to sheep-pox than other breeds. On the other hand, Chaumier (1905) inoculated a calf, a goat and an ass with sheep-pox material. He obtained no result on the calf, but goat and ass were both positive and transference to calf and children gave typical vaccinia. He also vaccinated or revaccinated sixty children with original sheep-pox material with success and found subsequently that immunity to normal vaccinia had been produced. Voigt (1909) obtained some equivocal results by inoculation of calf, goat and rabbit, but had misgivings that the experiments were not free from contamination with vaccinia. Roncal (1915) came to the conclusion that sheep inoculated with calf vaccine were not entirely protected, but admits that on the whole good results follow its use. He failed to inoculate a calf with sheep-pox, but succeeded with a goat, and found that the material after passage through a goat when inoculated into sheep gave better protection than the same material after passage through the calf. Gins (1919) inoculated rabbits cutaneously with sheep-pox and by repeated passage to the fourth rabbit produced material which, transferred to a calf, gave typical confluent vesiculation. He also showed that inoculated on rabbit cornea histological changes were set up which, after further passage became similar to, and finally identical with, those produced in normal vaccinia. Toyoda (1924) confirmed Gins on these points. Direct transference of sheep-pox to calf he found difficult, but passage through the ass facilitated the change. Complete immunity in sheep, calf, and child, also rabbits and guinea-pigs, was given by sheep-pox against cow- and bird-pox ; on the other hand, original sheep-pox is with difficulty inoculated on the hen, but passage lymph more easily. Vaccinized sheep-pox inoculated on the hen gives only partial immunity against bird-pox.

Gins (1920) showed that sheep could be immunized against fatal sheep-pox by inoculation with crude calf vaccine undiluted with glycerin. There was no generalization of the eruption, the reaction remaining purely local. Glycerinized lymph produced only partial immunity. He argued therefrom that some of the original virulence and immunizing capacity was lost in the dilution with glycerin and that full strength was necessary to give protection. He also found that sheep could be immunized by inhalation of fresh calf lymph diluted with 1 in 20 saline, and suggested that this could be done on a large scale by confining a number of sheep in a room of which the air was impregnated with the inoculating material. Blanc, Melanidi and Stylianopoulo (1926[2]) also succeeded in transferring sheep-pox to goats, and found that they were thereby immunized against that disease, but that retransference to sheep again set up sheep-pox.

Kii and Kasai (1925) inoculated sheep-pox virus testicularly into rabbits ; transference to calf gave no result after the first passage, but after three or four set up typical vaccinia. They state, however, that sheep cannot be immunized against sheep-pox virus by cow-pox inoculation or by sheep-pox virus passed through rabbit testicles but they do not appear to have used undiluted lymph. Gins (1927), however, failed with intravenous and intratesticular injection of sheep-pox into rabbits.

Blanc, Caminopetros and Melanidi (1927) found that intracerebral injection of sheep-pox virus into rabbits set up an encephalitis. The virulence increased with passage and the third rabbit died on the fourth day. The brain emulsion is said to have given typical vesicles on the calf and on children.

Bozzelli (1925) takes the view that horses are refractory to sheep-pox and experimentally failed to transfer it, but Blanc, Melanidi and Stylianopoulo (1926[3 & 4]), although they failed with inoculation of the skin and mucous membranes, succeeded with subcutaneous injections, which produced specific local reactions. Material from these subcutaneous lesions retransferred to sheep gave rise again to sheep-pox.

There can be no doubt that these positive results outweigh the negative experiences previously mentioned and establish that sheep-pox can be transformed into vaccinia by passage through the goat, calf or rabbit.

Variolation of sheep. Very few experiments appear to have been made in the inoculation of sheep with human smallpox. Friedberger and Fröhner state that according to Küchenmeister and Tapper, human variola injected into the blood of sheep produces a general eruption. Bollinger mentions that Küchenmeister succeeded in inducing in a sheep a pock infection by placing on its head a shirt from a person ill with smallpox.

Gins (1919) found that the contents of a pustule from an unvaccinated child dead of smallpox inoculated on to the skin and nasal mucous membrane of a sheep set up in five days, at the site of inoculation on the skin, small pea-sized pustules. At the same time the temperature rose and remained above normal with some intermissions for twelve days. There was no generalization of the eruption and no constitutional symptoms ; unfortunately it was not proved whether immunity to natural sheep-pox had been conveyed by the localized eruption. This is the crucial experiment and it is very desirable that further investigations on these lines should be made.

If human smallpox sets up in sheep localized manifestations only, with no infective powers, and sheep-pox induces in human beings a similar process to vaccinia, it is difficult to see how human smallpox and sheep-pox can have any dependence the one on the other. Yet it has been asserted that sheep-pox disappeared from Prussia as smallpox decreased (Gins, 1927), and that sheep-pox is absent in lands free from smallpox and prevalent where smallpox is much in evidence. The evidence at present available strongly suggests that human smallpox and sheep-pox are very closely related ; each sets up in its own particular soil a similar virulent disease of high infectivity ; each, after passage through the calf (or rabbit), is transmuted into a localized and relatively innocuous affection, which can afford protection against either parent disease. The inference is that human smallpox and sheep-pox are either offshoots of some common root virus or that the one is derived from the other, and, in the process of time by adaptation to the particular species, the noxious features of the disease have become peculiar to that species and are not transmissible to other animals.

Presence of virus of sheep-pox in organs. Nocard (1894) stated that the blood in sheep-pox is non-virulent. Blanc, Melanidi and Stylianopoulo (1926) found virus in brain six days after clavelization of sheep. There was no nervous disturbance, but inoculation of the sheep with brain emulsion set up sheep-pox.

Ecthyma contagieux des lèvres de Moussu or *chancre du mouton.* This disease has been carefully studied by Aynaud (1921–3).

Cross immunity. Sheep-pox is inoculable into men, bovines, goats and rabbits, and by such passage is transmuted into vaccinia or the equivalent of cow-pox. In this form it protects against human variola and the various animal poxes with the exception of some strains of bird-pox. Sheep-pox itself will protect sheep, rabbits and guinea-pigs against cow-pox and bird-pox (Gins, 1919, 1920 ; Toyoda, 1924 ; and others).

Sheep-pox is of economic importance, as in countries where it is prevalent it causes severe losses amongst flocks. Efforts have, therefore, been made to give protection against the disease. Inoculation with sheep-pox direct was found dangerous, just as variolation was in man, since it tended to spread the infection. Inoculation with calf vaccine appears on the whole to have given fairly satisfactory results, but most French observers deny its efficacy. These discrepancies may possibly be due to the use of an insufficiently strong vaccine, as Gins's experiments suggest. The French use ovinized vaccine attenuated in one or another way (Conte and Duclert, 1899; Ducloux, 1913). Borrel (1903) used virulent material mixed with serum from hyperimmunized sheep. These methods, especially the last, appear to have given fairly satisfactory results, but for fear of introducing the disease they are only used in flocks already attacked. Gins's method of employing crude calf lymph would be free from this objection, but has not been tried on a sufficiently large scale to permit of definite conclusions being drawn. Ducloux and Cordier (1926) attenuated sheep-pox virus by admixture with formic aldehyde or ethanol and conferred permanent immunity.

Goat-Pox.

(Variola caprina ; Variole de chèvres ; Ziegenpocken.)

Description. Goat-pox is an epidemic disease, infectious, and spread also by contagion, characterized by febrile and constitutional symptoms and a papulo-vesicular exanthem affecting the skin and mucous membranes.

Ætiology. Goat-pox is not a common disease, and is limited to those countries where goats are kept in herds: Greece, Italy, Norway and Algeria are the countries most affected, but it also occurs in Spain and France.

Bollinger (1877) was of opinion that goat-pox was contracted from sheep- or cow-pox ; Hutyra and Marek (1922) also suggest sheep-pox as a possible cause. Others, as Nocard and Leclainche (1903), look upon it as a distinct disease peculiar to goats. Zeller (1920) described pustular stomatitis in goats, which he had observed in German South-West Africa; he considered the disease to be related to goat variola, but according to Blanc, Melanidi and Stylianopoulo (1926[1] and 1927) this malady was similar to the disease they had studied in Greece, and found due to the same virus as ecthyma of sheep. The same authors say that true goat-pox should also be distinguished from another eruptive disease known as goat variola which they consider to be a bacterial 'pyodermite'. In this disease one attack does not immunize against itself or against stomatitis pustulosa.

Frese (1921) described an outbreak of goat-pox in Berlin, but the origin could not be determined. Natural sheep-pox infection could apparently be excluded, but contamination from experimental sheep or cow-pox was a possible source.

It is often stated that goats running with sheep afflicted with sheep-pox never contract the disease and similarly that sheep do not become infected with goat-pox although in close proximity to it. In view of the confusion that has arisen as to what disease actually is goat variola and the difficulties of diagnosis this assertion requires further investigation.

Symptoms. The incubation period is stated by Garbuti and Reali (1905) to be naturally 6 to 7 days. In artificial skin inoculation reaction commences in less than 48 hours (Frese, 1921). The invasion period is marked by fever and the usual constitutional symptoms. Red papular blotches which become well marked nodules the size of peas, or larger, characterize the rash. These nodules, especially on the udders and teats, become vesicular, are umbilicated and resemble vaccine vesicles. The exanthem may be confined to the udders or extend over the greater part of the integument, where it is most apparent on the smooth hairless parts, invading the mouth and the nasal cavity, larynx and trachea, occasioning marked catarrh which may eventually lead to pulmonary complications. Except for these sequelæ, the course of the disease is generally favourable, the duration being about two to three weeks.

Pathological anatomy. Macroscopically the lesions are very like those of sheep-pox. Nodules are found in the lungs and liver ; the intestinal mucous membrane may be swollen, showing punctate hæmorrhages, and the mesenteric lymph glands may be enlarged. Histologically the appearances are those usually associated with pock formation. No peculiarities have been observed.

Transmissibility. There is no record of goats having been variolated with human smallpox, but goatherds have been known to acquire the disease in the form of vesicles on hands and arms (Hansen, 1890 ; Marcone, 1900). Goats are susceptible to vaccinia, the lesions being very similar to those on the calf. Gins (1919) by repeated passage on skin of rabbit developed vaccinia from goat-pox. Definite results were not obtained until the fourth rabbit, which gave the same appearances as when inoculated with virulent vaccine. Carried on to a calf, it developed well. On the rabbit cornea a distinct cloudiness was caused after the fourth day. Macroscopically the usual appearances associated with Paul's test could not be made out, but microscopically cell inclusions were found similar to Guarnieri bodies. These appearances were more marked in the second rabbit, and on the third macroscopic and microscopic appearances were not distinguishable from those due to inoculation with an active vaccine. Blanc, Melanidi and Stylianopoulo (1926[1 & 2]) state that goat-pox cannot be transmitted to rabbits, dogs, chicken or the human being. It is inoculable on sheep, producing sheep-pox, and this is retransferable to goats. These authors, following Nocard and Leclainche, regard goat-pox as a specific disease, but with close analogies to sheep-pox and vaccinia. Differing views such as these do not necessarily contradict one another. It does not appear that Melanidi and his colleagues in their animal inoculations used the repeated passage method which Gins found successful. There seems no doubt that goats suffer from a pock disease which may become epidemic with, in some instances, considerable mortality. The origin for the present must be considered undetermined, but the available evidence suggests a close association with sheep-pox and that the most likely cause is infection from sheep. That difficulties in transmission to other animals exist is only what would be expected from analogy with human variola and sheep-pox. It seems to be a characteristic property of those affections which display virulence and are freely communicable to others of the same species that time and repeated change of soil are necessary before the adaptation to a new and strange host brings about the typical eruptive process. This feature marks another point of difference between the first and other groups of pock diseases.

So far very few observations in the light of modern knowledge have been made on goat-pox, and it is much to be desired that further investigations should be undertaken to determine more definitely the origin, transmissibility and general relations of the disease.

Swine-Pox.

(Variola suilla ; Variole des porcs ; Schweinepocken.)

Description. Swine-pox is an eruptive disease affecting skin and mucous membranes of swine, similar to sheep-pox and goat-pox, and occurs in epidemics as well as sporadically. It is probably spread by infection (air borne) as well as by contagion, but this, owing to lack of observation, has not been definitely determined. Aynaud (1923) affirms that in this respect and in its exanthem it has an absolute analogy with human variola.

Ætiology. Swine-pox was described by Ruhling in 1772. He considered it identical with human smallpox and held that one attack conveyed immunity for life. It occurs chiefly in Southern Europe, particularly Roumania and Hungary. Of recent years it has seldom been seen in Germany or England. It affects chiefly young pigs. The origin has been traced to human smallpox from the straw of infected beds or from infected clothes (Viborg). In one instance more than forty pigs are said to have gnawed clothes impregnated with variolous material, which had been used to cover a cow. Eight to ten days later all the pigs developed pock disease. Also, it may be contracted from sheep-pox, the pigs occupying the same stables as infected sheep, or stables in which infected sheep have previously been stalled. Bollinger (1877) considered that the disease was probably derived either from cow- or sheep-pox.

Symptoms. The course of the disease is similar to that of sheep and goat-pox. After an incubation period of three to seven days there is fever with catarrh of the respiratory tract, conjunctivitis, and so forth. In a day or two, red spots and blotches appear on the thighs and abdomen, head, snout, &c., most marked where the hair is scanty. In two or three days these spots become vesicular, then pustular, and eventually dry up and exhibit brown scabs. These pocks are often larger than those of sheep or goats. The disease may be mild with only a slight eruption, or severe with the exanthem widespread and sometimes confluent. The eruption may extend to the mucous membranes of mouth, nose, pharynx, trachea and bronchi, where it is apt to set up pulmonary complications which are often fatal. Isherwood (1926) gives the mortality as 20 per cent. and upwards.

Pathological anatomy. Few observations have been made on swine-pox. The large size of the pocks has been noted, and the occurrence of hæmorrhagic pocks is not uncommon. The colour of the pocks is a bluish black, and gangrene is fairly frequent, giving rise to the variety variola gangrenosa. Nodules may be found in the lungs and intestines similar to those of goats. Histologically, Heller has noted in the corium a remarkable increase of eosinophile leucocytes.

Transmissibility. Pigs are susceptible to vaccinia, the lesions produced being similar to those of sheep and goats. Gerlach states that swine-pox can be conveyed experimentally to goats and from them back to pigs. It is also reported to have been contracted by man (Friedberger and Fröhner, 1908).

Chauveau (1866) transmitted cow-pox to pigs, and Koch (1887) swine-pox to calves. Quasinsky inoculated sucking-pigs with sheep-pox and horse-pox with negative results.

Gins (1927) obtained some material from a case discovered in a slaughterhouse in Berlin, the origin of which was unknown, and by repeated passage on rabbit skin in the same manner as with sheep-pox and goat-pox was able to produce typical lapine on the fourth rabbit. From the sixth rabbit it developed an active lymph on a calf and also on a child. On the rabbit cornea the results were not so definite, up to the second passage neither macroscopic nor microscopic appearances were conclusive and at the third passage a severe specific infection was set up with rupture of the cornea, as if the inoculation had been made with virulent undiluted calf vaccine.

Horse-Pox.

(Equine variola ; Stomatitis pustulosa contagiosa ; Variola equina ; Pferdepocken.)

Description. Horse-pox is a sporadic non-infectious disease spread by contagion only, and characterized by a papulo-vesicular eruption affecting the general integument and mucous membranes. Usually it is accompanied by but slight febrile constitutional disturbance.

Ætiology. Jenner (1798) ascribed cow-pox to inoculation from ' grease ', a disease of the heels of horses. Loy (1801) was the first to realize that the term ' grease ' covered at least two diseases, one a specific communicable disease and the other a name casually applied to any running sore of the heels arising from fissures, eczema and so forth. Loy was successful in inoculating cows and children with horse-pox, producing typical vaccinia. Jenner thereafter used equine lymph for some time. Sacco (1803) confirmed the horse-pox theory and proposed the term *equine* as a substitute for *vaccine.*

From 1834 onwards French authors recognized that horse-pox was more than a localized affection of the heels, and comprised an eruption affecting the mucous membranes of the mouth, nose and genitalia, as well as the general integument (Lautour, 1834; Dard, 1840; Bouley and Patte, 1843; Dayot, 1850). Eggeling and Ellenberger (1878) and Friedberger (1879) made successful inoculations with material from the buccal eruption on to horse, ox, sheep and man, and gave it the name of stomatitis pustuleuse contagieuse, but considered it a separate disease from horse-pox.

In 1860 an outbreak occurred at Rieumes. This was investigated by Lafosse (1865), who successfully inoculated cows with the lesions, and from the cows, children. Bouley (1862) again studied the subject and described the multiple aspects of the disease. He gave it the name of horse-pox. At another outbreak at Rieumes, Peuch (1880) found the disease affecting the genitalia of brood mares and stallions and concluded that it was transmitted during service. Roll (1881) described the whole affection as one disease and states that Bassi in an examination of fifty cases only twice found it confined to the fetlocks; mostly the eruption was in the nose and mouth.

Occurrence. Horse-pox is an uncommon disease in this country. It is found sometimes on farms amongst the horses, when cows are affected with cow-pox, and occasionally in stud farms. It appears to have been more common in France during the latter part of last century. Its origin is as obscure as that of cow-pox. It is readily conveyed from horse to horse by grooms or farriers and probably from cows by attendants who have milked them. It is stated also that it may be contracted from human vaccination (Tintireanu, 1897), but the evidence of this is generally indefinite. Berger [quoted by Blanc, Melanidi and Stylianopoulo (1926^1)] is said to have observed natural infection of horses by sheep-pox on two occasions, but Bozzelli (1925) thinks this improbable.

Symptoms. After an incubation period of some four to six days, slight fever and malaise precede the eruption. This may occur on the skin, especially the flexures of the joints and more particularly the heels, and on the buccal, nasal, conjunctival and genital mucous membranes; sometimes it is localized to one or another of these sites, sometimes it extends to several. The course is nearly always benign and recovery is generally complete in two to three weeks.

Pathological anatomy. Histological examination of the lesions in horse-pox have been made by Eggeling and Ellenberger (1878) and Klose (1921). Nothing peculiar or characteristic was noted.

Transmissibility. Horses are susceptible to vaccinia and have been used for the production of vaccine lymph for human vaccination. Attempts to variolate horses by subcutaneous and intravenous injection have been made by Berthet (1884); Chauveau (1866); Warlomont (1883) and others. In some instances an eruption was produced, but the reports are not clear as to whether the eruption was analogous to variola or to vaccinia. No similar attempts appear to have been made in recent years. It is very desirable that a series of well-devised experiments should be carried out to determine more precisely the relations of the horse to inoculated variola.

Blaxall (1903) showed that material from vesicles from the mucous surface of the mouth of a horse suffering from horse-pox gave rise to vaccinia on the calf, two passages being necessary to effect full development. The animal inoculated was found subsequently to be completely immune to normal vaccine lymph.

De Jong (1916) confirmed the identity of stomatitis pustulosa contagiosa in horses with horse-pox by transference to cattle and rabbits with the production of vaccinia. He also found that retransference to horses gave rise to stomatitis pustulosa contagiosa. Inoculation of rabbit cornea occasioned typical vaccine appearances, and Guarnieri bodies were demonstrable in the corneal lesion. From cattle, inoculation of the passaged horse-pox gave typical vaccinia on children with subsequent complete immunity against normal vaccine. Further confirmation came from Toth (1922) and Zwick (1924). The last author passed the virus through the calf to the hen, sheep, rabbit (skin and cornea), dog, swine, and finally men. Tested with vaccine lymph later all were found to be immune except the hen, which showed only partial immunity. Van Heelsbergen (1920) inoculated a hen with horse-pox with success and set up stomatitis pustulosa contagiosa in a horse by inoculating it with hen-pox.

Cow-Pox.

(Variola vaccinæ ; Vaccinia ; Vaccine ; Kuhpocken.)

Description. Cow-pox is a specific eruptive disease affecting chiefly milch cows, spreading by contagion and not by air-borne infection. It is seldom observed in male animals or young heifers. The eruption is localized almost exclusively to the udder and teats, causing little general disturbance.

Ætiology. The origin of outbreaks of cow-pox has not yet been made clear. Jenner (1798) attributed cow-pox to the transference to the teats and udders of cows during the process of milking, by the hands of attendants, of a disease occurring in the heels of horses, known to him as ‘ grease ’ ; probably in some at least of the instances investigated by him he was right. When outbreaks of cow-pox occur, it is not uncommon to find the horses on the same farm affected at the same time, but which was the prior affection is generally difficult to determine. Horse-pox, however, is certainly not the only source of cow-pox. Cow-pox appears to have been much more prevalent in the eighteenth and nineteenth centuries than of recent years. This has been ascribed to the greater prevalence of smallpox and also of human variolation when this was common, the suggestion being that there was a close relation between the two diseases, and that the frequent occurrence of outbreaks of cow-pox was directly associated with the prevalence of smallpox. A similar interdependence has been suggested between sheep-pox and smallpox.

That cows could be infected by people suffering from smallpox has often been asserted, but concrete instances are not easily found; one such, however, was communicated to Jenner by Dr. Waterhouse, of Cambridge, Mass. In this a number of cows were milked by persons in all stages of smallpox, with the result that the cows had an eruptive disorder on their teats and udders, very like smallpox pustules. Such an origin or a similar occurrence arising from infection from human variolation must be looked upon as possible, but bearing in mind the known difficulty of artificially inoculating cows or calves with variolous matter it cannot be assumed to be a common cause.

Human vaccination is often put forward as a mode of infection capable of starting the disease, and several instances have been noted where cow-pox has broken out after recent vaccination of a milker or attendant. This, again, is no doubt a possible cause, but at best it would only account for a small proportion of the outbreaks. It is to be remembered that cow-pox was well-known long before the introduction of vaccination and apparently more prevalent then.

In an ordinary outbreak of cow-pox the cause most commonly found is the importation into the herd of new cows either with the lesions upon them or incubating the disease, and sometimes it is possible to establish this method of introduction, but by no means always. On the other hand, cow-pox has been known to occur on isolated farms where there has been no introduction of new cattle, where no horses are kept, where there have been no cases in the neighbourhood of variola or human vaccinations for some time past, and where nothing, in fact, can be found suggestive of the cause of the outbreak. Ceely (1840), who investigated numerous outbreaks of cow-pox, found the same difficulty and says that in all the cases he has noticed he could discover no other source than spontaneous origin.

Symptoms. In the natural disease, that is the disease uninfluenced by the handling of milkers, there is an incubation period of 3 or 4 days, which, according to Ceely, may extend on occasion to 5 or 8 days. There follows some slight inflammation and tenderness of the udder and the development of papules, perceptible at about pin-head size, rapidly growing larger to form firm reddened nodules with indurated bases. In 3 or 4 days some nodules may become distinctly vesicular, surrounded with an areola; others do not show the vesicular character so plainly, but remain red and firm though often showing a central depression. Jenner laid stress on a bluish coloration of the lesions; this does not seem a definite characteristic, however, but one dependent on the colour of the skin; ordinarily the lesions show the dull reddish coloration of mild inflammation. The lesions are situated on the teats, parallel with the long axis, in the shape of beans or long ovals, and on the base of the udder, where they are commonly circular. They vary in size from a pea to a large bean or a florin and they may be met with at different stages on the same animal. There may be few, only one or two, or as many as twenty or more, and some of these may be confluent, forming large irregular patches. From the vesicle, on rupture, exudes a thick viscid yellowish fluid. The vesicles take 6 or 7 days to reach maturity, then they begin to dry up, forming hard brownish or bluish crusts which separate in another 5 or 6 days, leaving an oval or roundish scar of no special appearance. The normal course of the disease occupies from three weeks to a month. Natural cow-pox would be but a slight ailment in cows, were it not for the mechanical irritation set up by the frequent handling of the sores in the process of milking. These necessary manipulations undoubtedly aggravate the lesions and thereby the general illness; moreover, they tend to spread the disease from animal to animal. When this occurs it is known as the casual form of the disease, and then the eruptive appearances are somewhat different. Vesicles are not commonly seen, the surface of the nodule being rubbed off, leaving a raw ulcerated surface with a hard indurated base.

Generalization of the eruption is very rare, but instances are said to have been observed by Strebel (1898), Dupuis (1889), and others. Pocks appear on the head, at the base of the horns, and on various parts of the general integument. There appears to be little or no aggravation of the general symptoms. There is no record of the mucous membranes of the mouth and air passages being affected.

Pathological anatomy and histology. No special investigations of a histological nature appear to have been made on original cow-pox; there is no reason to suppose that the lesions would exhibit any marked difference from those of inoculated cow-pox on the calf, which have been the subject of much research as indicated in Chapter VII (see Weigert, Unna, Councilman and Mann).

Transmissibility and immunity. Cow-pox is readily transmitted to man, setting up lesions especially on the hands of the milkers. The parts particularly affected are the dorsal surface, especially between the thumb and forefinger, the flexures of the joints or any aspect of the fingers, wrist and lower arm. From the hands the infection is readily conveyed to the face, eyes, mouth, ears and so forth, and not infrequently serious effects are brought about, especially if the eye is affected. The lesions always remain localized; there is no generalization of the eruption.

Cow-pox in man may recur in the same individual. Jenner speaks of a man who had three attacks and Ceely saw it more than once in the same person. Both observers say that it may occur after smallpox, and Ceely also adds after vaccination 'at periods of from 2 to 15 years'.

In 3 outbreaks of cow-pox the writer finds that 27 persons were affected, who were all stated to have been vaccinated in infancy. These included 2 children under 5 years of age; in their case it was suggested that the infection had been brought about by insect bites, as they had had no direct association with the affected cows. One of the 27 had been revaccinated 7 or 8 years previously, as recorded by Reece (1921), who also observed that of 44 milkers examined, of whom 23 suffered from cow-pox, most of them had been vaccinated in infancy. In the great majority of instances an interval of some 20 years had elapsed between the cow-pox and the previous vaccination, and it is reasonable to assume that immunity had waned.

Variolation. Cows or calves can be variolated (see Chapter VII) but only with difficulty. It is more easily effected through the intermediary of another animal, such as the monkey.

Transmission to other animals. Cow-pox is readily inoculable on most of the mammalia and on fowls and pigeons, and from them it can be retransferred to the calf, setting up the same disease, cow-pox or vaccinia. The animal pock diseases vary considerably in their transmissibility to the calf. Success is usually obtained with horse-pox, fowl-pox, and human cow-pox, though horse-pox and fowl-pox may require repeated passage before yielding typical vesicles ; this, however, may be due to attenuation of the virus through using unsuitable material, such as old crusts. On the other hand, sheep-, goat- and swine-pox do not take on the calf readily, but only after passage through some other animal, such as the monkey, rabbit or ass ; the resemblance here to variola is noteworthy.

Nature of the Virus of the Various Animal Pock Diseases.

These viruses are in the same position as those of variola and vaccinia. None has as yet been identified. Guarnieri bodies have been described in the lesions set up by inoculation of sheep-, goat- and swine-pox on the rabbit cornea and in sheep-pox skin lesions, Gins (1927). Toyoda (1924) found them in lesions produced by inoculation of fowl-pox, De Jong (1916) in horse-pox inoculations of rabbit cornea, and it is understood that granules similar to Paschen bodies have been seen intracellularly in fowl-pox lesions, but nothing definite has so far been established.

Filterability.

Far fewer attempts to filter the animal poxes have been recorded than is the case with variola-vaccine virus, but sheep-pox has been claimed as a filter-passer by Borrel (1902) using a Chamberland filter; fowl-pox by Marx and Sticker (1902) using a Berkefeld, Ledingham (1926) in one instance, and others ; pigeon-pox by Juliusberg (1904) and others ; and swine-pox by Poenaru (1913). Findlay (1928) succeeding in passing fowl-pox through a Berkefeld filter, but failed with vaccinia virus ; with vaccinia lesions from the chick he obtained one positive result in eighteen attempts.

Action of Physical and Chemical Agents.

So far as has been determined the viruses of other animal poxes show similar reactions to variola and vaccinia. They withstand drying and very low temperatures for long periods ; moist heat is rapidly destructive ; the lethal point of heat is from 55 to 65° C., varying with the length of time of exposure, and resistance to the action of glycerin at low temperatures is greater than that of ordinary bacteria. No distinctive reactions have been noted in regard to chemical disinfectants, but Bridré and Donatien (1921) state that they consider the virus of sheep-pox more sensitive to heat, light and antiseptics than vaccine virus.

Artificial Culture.

No definite success has attended attempts to cultivate the virus of the animal poxes outside the body, with the exception of those dealing with vaccinia or cow-pox virus, and fowl-pox (Findlay, 1928).

Summary.

The animal pock diseases viewed as a whole show a strong resemblance one to the other ; one, however, cow-pox, displays a decided differentiation in certain aspects.

1. All present one feature in common, a characteristic eruption on the skin.
2. In cow-pox this eruption is purely local ; in all the others it is, or tends to be, more or less generalized. This tendency is not so pronounced in the fowl and horse as in sheep, goats and swine, but it is sufficiently marked to differentiate the disease in those animals from cow-pox.
3. In all the animal poxes, except cow-pox, the eruption attacks the mucous membranes, especially those of the upper respiratory passages, either alone or in conjunction with the skin. The importance of this extension of the disease is seen in the mortality. In human variola much of the fatality is occasioned by respiratory involvement or by septic invasion from the affected mucous membranes. In sheep, goats and pigs such involvement is the cause of much of the mortality, and in fowls it may be said to be wholly responsible. In horses, fatality is uncommon, but when it occurs it is almost always due to this cause. In cow-pox there is no similar lesion and no fatality.

Infectivity varies considerably in the different poxes. Human small-pox is probably the most infectious, followed closely by sheep-pox. Goat and swine-pox appear to spread less readily, though this perhaps may be due to meagreness of infective material. Horse-, fowl- and cow-pox are probably spread only by direct contact with infective material.

From these considerations it is obvious that the cow or calf is, par excellence, the most suitable animal to use in reference to human vaccination, as on this animal the harmful properties of the other animal poxes do not materialize. In the possession of these peculiarities the rabbit seems to be close to the calf.

The virus derived from each of these diseases, with the possible exception of fowl-pox, in regard to which different observers have obtained contradictory results, seems to have the same properties ; each virus is inoculable on each of the animals liable to pock disease, and on some others such as rabbit ; each virus sets up lesions in which cell inclusions, such as Guarnieri bodies, or similar effects, such as molluscum bodies, can be found; each virus can be reduced to a standard type, that of vaccinia by passage through suitable animals, and each immunizes against all the others.

That smallpox and all the animal poxes, excepting possibly fowl-pox, have a very close relationship is certain. Indeed the viruses of these diseases may be looked upon as identical, except for such variation as has been induced by adaptation to the special animal organismus with which each is associated, and their general similarity points to the conclusion that they have evolved from one common root stock.

If the processes of disease develop like all other evolutionary processes from the elementary and simple to the complex and elaborate, it is logical to assume that variola and the various pock diseases have been evolved from the simplest and least nocuous disease, that is, cow-pox.

If the view be accepted that cow-pox is, so to speak, the bedrock virus, it becomes intelligible how variola and the animal poxes can by passage through the cow or calf revert to the primal type, that is, vaccinia, and how vaccinia can protect against the more complex forms, culminating in human smallpox, which have all originated from it.

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CHAPTER X. HERPES, ZOSTER, VARICELLA.

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Herpes.

Clinical Features.

HERPES is characterized by a vesicular eruption appearing on the skin and mucous membranes. The vesicles are arranged in clusters on a more or less erythematous base and their appearance is heralded by itching, pricking or burning sensations. Occasionally the eruption is preceded by definite nerve pain as in zoster (Nicolau and Poincloux, 1924). The vesicles appear within a few hours of the onset of premonitory symptoms and soon dry up, forming crusts which fall off leaving no scar. The herpetic eruption affects particularly certain cutaneous areas such as the skin around the mouth, on the lips, around the nostrils, on the prepuce and on the buttocks. The mucous membranes of the mouth, nasopharynx, urethra and vagina may also be the site of the eruption, though here typical vesicles are rarely seen. These localizations have given rise to such terms as herpes labialis, h. buccalis, h. genitalis, &c. The cornea also is not infrequently attacked, giving rise to an herpatic keratitis of varying severity. Clinically, herpes may be divided into two groups: (1) idiopathic (h. febrilis) and (2) symptomatic, the vast majority of cases belonging to the second category. The occurrence of herpes in the course of such diseases as coryza, pneumonia, cerebrospinal meningitis, malaria, influenza, infectious jaundice, typhus and as the result of protein shock therapy or vaccine administration is well known. Also in those individuals who are subject to herpes, emotional strain, worry, digestive upsets and menstruation may precipitate an attack.

Epidemiology.

Experimental work has demonstrated beyond doubt that the herpetic vesicle contains a specific agent by means of which the condition can be reproduced and passed in series, and there is clinical evidence that herpes is contagious and may occur in epidemic form. In the vast majority of cases, however, infection from without can be discounted, and one must either suppose that in such cases the individuals are carriers of the virus, ready to be lit up by one of the many predisposing causes, or else assume that the virus can arise *de novo* as a result of some change produced in the cells of dermis or epidermis. The question is of fundamental importance, for the correct answer to it will provide the solution to the problem of the nature of this group of disease agents. It must be admitted that the view which would make the herpes virus both the cause and the result of the disease is difficult to accept and unsupported by positive evidence, whereas it has been demonstrated that herpes virus is present in the saliva and conjunctival sac of normal individuals (Levaditi, Harvier and Nicolau, 1922; Busacca, 1924–5). Should the virus be shown capable of cultivation in a lifeless medium the question would be settled.

Pathology.

Lesions of the skin and cornea. With the exception of those changes which are due to the trauma of inoculation, the lesions of the skin are essentially similar in spontaneous and experimental herpes and find a close parallel in the vesicular lesions of varicella and zoster. The affected cells of the epidermis are swollen and show the ballooning and reticular degeneration which are a feature of this type of lesion. As a result of amitotic nuclear division some of the epithelial cells are transformed into large multinuclear cells (Mariani, 1924). The affected epidermis becomes infiltrated with leucocytes (polymorphonuclear and mononuclear) and a serous exudate dissociates the cells and collecting beneath the stratum corneum forms the vesicle. The adjacent dermis shows vascular engorgement and leucocytic infiltration, changes which, according to Teissier, Gastinel and Reilly (1926) precede those in the epidermis. The changes in the corneal lesion are very similar to those met with in the skin, the differences being due to the peculiar histological structure of the former tissue. In the rabbit lesions are found in the nerves of the cornea, retina and ciliary body and the optic nerve, indicating the route by which the virus reaches the brain.

Lesions of the central nervous system. The microscopical changes produced by the herpes virus vary in intensity and localization with the virulence of the strain and the route of infection. Thus after corneal inoculation in rabbits the maximal incidence is in the neighbourhood of the trigeminal nucleus on the same side, whereas after intracerebral inoculation it is in Ammon's horn (McClellan and Goodpasture, 1923). Lesions of the cord occur most commonly after cutaneous or testicular inoculation. Wherever they occur the lesions have the same histological structure. The meninges are infiltrated with lymphocytes, adventitial and plasma cells, which extend along the vessels in the form of well-marked perivascular cuffs (Levaditi, Harvier and Nicolau, 1922). Should the process run a more rapid course the infiltration is more intense and almost entirely polymorphonuclear (Levaditi, Harvier and Nicolau, 1922). The vessels of the affected zones are dilated and frequently plugged with polymorphonuclear leucocytes, which pass out from the vessels and infiltrate diffusely the adjacent grey matter. Injury to nerve-cells is evidenced by tigrolysis and by their staining more deeply with eosin. The chromatin reticulum of their nuclei breaks up and collects at the periphery, the centre of the nucleus being occupied by eosin-staining material. Later, neuronophagia occurs. As Flexner and Amoss (1925[1]) point out, it is the changes in the nerve-cells which are of importance, for they alone are responsible for the symptoms observed.

Inclusion bodies in herpes. Of the various inclusion bodies described, those of Lipschütz (1921) are of the most significance. These are acidophil intranuclear bodies, which, though small at first, increase in size and coalesce, finally occupying the greater part of the nucleus. They occur in the epithelial cells of the corneal and skin lesions and in the nerve-cells and glial cells of experimental herpetic lesions of the central nervous system. They have also been observed in herpetic lesions of the liver, testicle and ovary (Goodpasture and Teague, 1923). The nature of these bodies is a matter of dispute. They are not specific, for precisely similar ones occur in zoster, chicken-pox and Virus III lesions, and the general tendency to regard them as a peculiar form of degeneration is most probably right. For a detailed study of these and other cellular inclusion bodies described in herpetic lesions the reader is referred to an article by Cowdry and Nicholson (1923). (See also Chapter II.)

Evidence that Herpes is due to an Ultramicroscopic Organism.

This may be summarized as follows :

(1) No formed incitant can be demonstrated by microscopical or cultural methods in the vesicle fluid.

(2) Typical lesions can be produced in susceptible animals by the cutaneous inoculation of vesicle fluid and the condition passed in series.

(3) The agent present in the vesicle fluid can pass through filters which retain the ordinary bacteria.

(4) The histology of the herpetic lesion is characteristic of infection with this type of disease agent.

Nature of Virus.

Resistance to various physical and chemical agents. Experiments designed to test the effect of various agents on a filterable virus are of doubtful value because the presence of much foreign protein may interfere with the reaction and because of the impossibility or great difficulty of separating virus and reagent after the given period of contact. Generally speaking the behaviour of herpes virus to the various antibacterial agents is not incompatible with it being of bacterial nature. The resistance of herpes virus to glycerol is variable. Some strains retain their activity for a year or more in 50 per cent. glycerol, others become inactive in a few weeks ; this would appear to be a function of initial virulence (Flexner and Amoss, 1925[1, 2 & 3]). Levaditi and Nicolau (1922) observed that a herpes virus kept in glycerol might lose its power of producing skin lesions, whilst retaining intact its virulence for the brain. This is in keeping with the author's experience that skin strains soon become inactive when stored in glycerol. A similar dissociation of the two activities can be brought about by heat, for Vegni (1922) and Mariani (1924) found that on exposure to 56° C. the keratogenic activity was lost before the encephalitogenic. Apparently oxidation may do the same, for Perdrau (1927) found that a rabbit's brain virus kept best at a low oxygen tension, whereas the author's observations (unpublished) would point to this factor being unimportant in the preservation of skin strains. The phenomenon appears worthy of further investigation. The degree of dilution which herpes virus will stand without loss of activity varies with the different strains. Rabbit's brain strains show titres from 1 in 1,000 (Levaditi, Harvier and Nicolau, 1922) to 1 in 1,000,000 (Le Fèvre de Arric, 1925); skin strains are rarely active beyond a dilution of 1 in 10,000.

Centrifugation. Levaditi, Harvier and Nicolau (1922) could not demonstrate any appreciable concentration of herpes virus (rabbit's brain suspension) after prolonged centrifugation at 3,000 r.p.m. The author (1927), working with a guinea-pig skin strain (diffusate), showed that the virus could be thrown down to an appreciable extent in two hours at 5,000 r.p.m. The possibility of different degrees of dispersion of the virus being accountable for this divergence of findings should be considered.

Filtration. Although the available evidence indicates that herpes virus can pass through the more porous Chamberland and Berkefeld candles (Levaditi and Harvier, 1920; Blanc, 1921; Flexner and Amoss, 1925[2]; McCartney, 1925) it does so with difficulty, suffering considerable loss in the process. How far this difficulty is due to the size of the virus particles is not known. No attention has been paid to the electrical charge carried by the virus or to the pH at which the experiment was performed, and, with the exception of McCartney (1925), workers in this field have hardly considered the part played by tissue proteins and lipoids in these filtration experiments. Levaditi and Nicolau (1923) claimed that this virus will pass through collodion membranes which are only slightly permeable to serum-proteins, and Zinsser and Tang (1927) from ultrafiltration experiments estimate that the virus particles are between 20 and $100\mu\mu$ in size. These experiments have little, if any, significance, for the results were inconstant, the experiments were not performed quantitatively, and the membranes were tested for gross defects only. Working with a skin strain the author (1927) failed to obtain evidence that herpes could pass through collodion membranes which allowed the passage of the bulk of the serum-proteins, a finding which is supported by his centrifugation experiments. His results led him to the conclusion that the virus is considerably larger than previous workers have thought.

Cultivation. Parker and Nye (1925) have shown that the virus of herpes can be cultivated *in vitro* in the presence of living tissue-cells (rabbit's testicle). Interesting though this observation may be, it throws little light on the nature of the virus. It is all important to know whether it can be made to multiply in a lifeless medium, and though, so far, efforts in this direction have proved abortive, this line of investigation should be thoroughly explored.

Transmission Experiments.

In man. Both auto- and hetero-inoculation by the cutaneous route succeed in man, the lesion produced resembling spontaneous herpes in all respects. After a few passages an individual usually becomes insusceptible to his own strain of virus, though still remaining susceptible to foreign ones (Teissier, Gastinel and Reilly, 1926). The exanthemata during the eruptive phase, as well as mumps and chicken-pox, appear to render an individual insusceptible to herpes virus (Tureau, 1924), whereas cases of encephalitis lethargica respond in an exaggerated manner (Teissier, Gastinel and Reilly, 1926).

In other animals. The rabbit, guinea-pig, rat and mouse are all susceptible to herpes virus. Contrary to what was thought the insusceptibility of the dog, cat and pigeon, though of a high order, is not absolute (Remlinger and Bailly, 1926). Of the monkeys *M. cynomolgus*, the chimpanzee (Levaditi, 1926) and the callithrix monkey (Teissier, Gastinel and Reilly, 1925) have been found receptive; as in man, skin and corneal inoculation does not result in involvement of the central nervous system. The rabbit and guinea-pig are undoubtedly the experimental animals of choice, and of these the former is the more susceptible. Cutaneous and corneal inoculation of the rabbit with weak strains results in a local reaction only, but with virulent strains a spread to the central nervous system occurs frequently, particularly after corneal implantation. The encephalitic syndrome in the rabbit is characterized by convulsions, opisthotonos, circus movements, loss of co-ordination, myoclonia, nystagmus, salivation, paresis and paralysis (Levaditi, 1926), but not infrequently the picture presented is atypical (Remlinger and Bailly, 1926). The mortality varies with the strain employed. Inoculation of the guinea-pig on the cornea, hairy skin, or in the pads, gives rise to local lesions; only with strains of exceptional virulence does the brain become involved after corneal inoculation (Flexner, 1928). A small percentage of guinea-pigs inoculated in the plantar skin develop a myelitis which does not ascend higher than the mid-dorsal region (Rose, 1925–6). Given a strain of sufficient virulence brain to brain passage can be realized in the guinea-pig just as in the rabbit (Flexner, 1928). There seems no doubt that brain passage of a herpes virus results in loss of affinity for the skin and cornea and vice versa.

Immunity.

It is doubtful whether any immunity is developed in man as the result of an attack of herpes; at most there may be a local insusceptibility following recovery, an ephemeral state which does not occur with any regularity. In other animals recovery from infection by whatever route is associated with a general immunity which is well established by the fifteenth day and lasts at least four months. Following a localized corneal infection in the rabbit the general immunity developed may be of low grade and shortlived: sometimes the immunity is purely local (Perdrau, 1925). Immunity of the uninoculated cornea is slow in becoming established—one month according to Doerr and Schnabel (1921)—and may not be of as high degree as in the inoculated eye. Perdrau (1925) has observed that intracerebral inoculation of a partially immune rabbit results in an atypical encephalitis, lethargic in type, and Zinsser and Tang (1926) are of the same opinion. When the nature of this general immunity is in question unanimity is no longer found. Levaditi (1926) considers it a purely tissue one, but since the serum of recovered animals has been shown to possess specific viricidal activities (Zinsser and Tang, 1926; Bedson and Crawford, 1927) and that guinea-pigs can be rendered passively immune (Bedson and Crawford, 1927), Levaditi's contention does not seem justified. There is evidence that in the case of vaccinia a mixture of virus and antiserum, although neutral when tested in the skin, might not be neutral when tested in another tissue such as the testicle (Andrewes, 1928), and the same might apply to a mixture of herpes virus and antiserum when tested in the skin and brain. As well as being a possible explanation of the divergence of findings referred to, it is of itself a most interesting phenomenon and worthy of investigation. The mode of action of a viricidal serum also requires elucidation, and as far as herpes virus is concerned Bedson (1928) has obtained evidence which suggests strongly that herpes virus unites with its specific antibody *in vitro*. Unless such a union does occur in the test-tube it is difficult to explain the specific complement fixation which has been demonstrated with a number of viruses. In the case of herpes virus Takaki, Bonis and Koref (1926), using a kokto antigen, obtained specific complement fixation enabling them to distinguish herpes from other viruses.

Zoster.

(Zona; Herpes Zoster.)

Clinical Features.

Zoster is characterized by a vesicular eruption preceded usually, and always followed, by pain, which is often intense. This pain is referable to one or more of the nerve-roots and the eruption is limited to the cutaneous areas supplied by the involved nerves. The clear hemispherical vesicles surmount erythematous plaques of irregular contour. The vesicles rapidly become pustules, and dry up in the course of a few days, forming crusts. As a rule the eruption is unilateral, but occasional cases with aberrant vesicles occur, and generalized zoster has been described. The lymph-glands draining the involved cutaneous area are enlarged. Zoster can be divided into two main groups: (i) idiopathic and (ii) symptomatic. In idiopathic zoster there are commonly prodromal symptoms such as slight pyrexia, malaise and local sensations of itching and burnings. The symptomatic zosters occur in the course of certain infections (syphilis, poliomyelitis, German measles, epidemic encephalitis), in intoxications (arsenic, bismuth, lead, alcohol, &c.), and as a result of damage to peripheral nerves, root ganglia and the cord (neoplasms, tuberculous foci, sciatica, trauma). There is no difference in either the appearance or the course followed by the eruption in the idiopathic and symptomatic zoster, and the underlying feature of them all is nerve damage. Relapse is extremely rare.

Epidemiology.

Although there is some evidence (Marinesco and Draganesco, 1923) that small epidemics of zoster occur, it does not appear that a case of zoster commonly gives rise to zoster in those in contact with it. There is, however, ever accumulating clinical testimony which points to cases of this disease giving rise to chicken-pox in susceptible individuals. Von Bokay was the first to be struck by this fact, and he postulated a common ætiological agent for chicken-pox and idiopathic zoster. It is impossible here to discuss in detail the steadily growing literature on this question, and the reader is referred to the following papers, where it is reviewed: Le Feuvre, 1917; Low, 1919; Ker, 1920; Roxburgh, 1927. It is interesting, however, to note that of the various associations of the two diseases which might be expected had they a common ætiology, that of chicken-pox following zoster within the incubation period of the former disease alone occurs with sufficient frequency to make chance coincidence improbable.

Clinical evidence has of late received the support of experimental findings. Netter and Urbain (1924, 1926) claim that an antigen prepared from the crusts of zoster lesions gives equally good complement fixation with sera from zoster and chicken-pox convalescents, the converse of this being also true, and Kundratitz (1925) has found that, whereas children who had neither chicken-pox nor zoster could be inoculated cutaneously with zoster vesicle fluid, those who had had chicken-pox were refractory. The foregoing remarks have reference to idiopathic zoster; what of the symptomatic cases? There is evidence (Netter and Urbain, 1924) that arsenical and bismuth zosters are due to the same virus as the idiopathic ones, and it seems not unreasonable to suppose that the same holds true for other symptomatic zosters. The writer puts forward the hypothesis that the virus of all types of zoster is the same as that of chicken-pox, and that a number of individuals are carriers of this virus in the central nervous system. The dormant virus is lit up by nerve injury (spinal cord, root ganglia or peripheral nerves) and reaching the skin by way of the affected nerves produces an eruption in the area of skin supplied by them. Infection from zoster usually takes the form of chicken-pox because the blood-stream is more readily invaded from the mucous membrane of the upper respiratory passages than is the central nervous system.

Evidence that Zoster is due to an Ultramicroscopic Organism.

No causal bacterium can be demonstrated in zoster material; in fact, the vesicle fluid from early lesions is culturally sterile. Kundratitz (1925) claims to have transmitted the disease to normal children with zoster vesicle fluid. To this evidence must be added the presence of acidophil inclusion bodies (intranuclear) in zoster lesions.

Pathology.

Most important, perhaps, are the changes in the central nervous system, the knowledge of which we owe principally to the classical investigations of Head and Campbell (1900). These changes occur most frequently in the posterior root ganglia and consists of hæmorrhages, perivascular cuffing and areas of mononuclear infiltration. The nerve-cells show tigrolysis and their nuclei are obviously affected. The acute phase is followed by one of regeneration with absorption of blood-pigment, neuronophagia and proliferation of capsular elements. Head and Campbell observed similar inflammatory changes in peripheral nerves, in some instances without lesions in the root ganglia. Wohlwill (1924), who has confirmed and extended the work of Head and Campbell, states that cases of zoster, idiopathic and symptomatic, show constant lesions of the primary sensory neurone, the posterior root ganglia being frequently, though not invariably, affected. In addition, Wohlwill finds that the cord is often the seat of lesions as well—a posterior poliomyelitis—the posterior horns, principally at their basis, being infiltrated with mononuclear leucocytes. Unlike poliomyelitis the neurons remain relatively intact. The cerebrospinal fluid in some cases show a lymphocytosis which may be considerable (Brissaud and Sicard, 1901 ; Chauffard and Frouin, 1902 ; and others). The skin lesions resemble those of herpes. Acidophil nuclear inclusion bodies—‘ Zosterkörperchen ’—are present in the cells of the vesicle epithelium, also in connective tissue-cells and capillary endothelium of the dermis (Lipschütz).

Nature of the Virus.

Nothing is known of the nature of the virus. Its filterability has yet to be demonstrated.

Transmission.

Experiments on man. Kundratitz (1925) inoculated 22 children cutaneously (21) and intradermally (1) with fresh vesicle fluid. Of these, 14 reacted with the development of local vesicles 9 to 11 days after inoculation, followed in two instances 4 days later by a generalized eruption indistinguishable from chicken-pox. Siegl (1927) reports the production of chicken-pox in 2 out of 6 children, 22 to 23 days after cutaneous inoculation with zoster vesicle fluid.

Experiments on laboratory animals. The majority of efforts to transmit zoster to laboratory animals have proved entirely fruitless (Cole and Kuttner, 1925). Some doubtful results have been recorded in the case of rabbits inoculated on the cornea with vesicle fluid (Lipschütz, 1925 ; Marinesco and Draganesco, 1923). A certain number of the inoculated animals developed a mild keratitis, which on histological examination showed the presence of acidophil nuclear inclusion-bodies. Attempts to pass the condition invariably failed.

Immunity.

Active immunity. Clinical observers are agreed that an attack of zoster confers an immunity which in the majority of cases is solid and lasting. The experimental work of Kundratitz (1925), indicating that chicken-pox confers immunity against zoster and vice versa, has already been referred to.

Serological reactions. Netter, Urbain and Weissmann-Netter (1924) found that the serum of convalescent zoster cases gave specific complement fixation with a saline emulsion of the crusts of zoster lesions, and mention has been made (Netter and Urbain, 1924, 1926) of the crossed fixation demonstrable with the sera of zoster and chicken-pox convalescents.

Varicella.

Clinical Features.

After an incubation period of 10 to 15 days the disease sets in with pyrexia, vomiting and pains in the back and legs. As a rule these symptoms are slight and may even pass unnoticed. Within 24 hours of onset the eruption appears on the chest and back ; in rare cases it may begin on the forehead or face. It consists at first of raised red papules which become vesicular in a few hours. The vesicular contents become purulent in 36 to 48 hours, and by the third or fourth day the pocks shrivel up, forming dark brown scabs, which fall off, leaving no scar. Fresh vesicles appear during the first two or three days of illness, so that by the fourth day individual lesions of varying degrees of maturation may be seen. Pressure, such as that exerted by a bandage or clothing, will determine an increased local incidence of vesicles (Rivers and Tillet, 1923). In the great majority of cases the disease runs an uncomplicated course and recovery without ill effects is the rule. Hæmorrhagic cases have been described and amongst the rare complications are nephritis, myelitis and encephalitis.

Epidemiology.

Chicken-pox would appear to be spread by both direct and indirect contact. It is essentially a disease of childhood, affecting principally the second to the sixth years of life. Perhaps the most interesting feature in the epidemiology of this disease is the relationship to zoster.

Pathology.

Chicken-pox being a benign disease there has been little opportunity for the study of its morbid anatomy and histology. The skin lesions, however, have been examined in some detail (Unna, 1900 ; Tyzzer, 1905–6, and others), but since they are essentially similar to those of herpes it is unnecessary to describe them. Tyzzer (1905–6) drew attention to the appearance of eosin staining bodies in the nuclei of affected cells, and these nuclear inclusion bodies have since been studied by Bertarelli (1909), Gins (1918), Lipschütz (1921), Simon and Scott (1924), Rivers (1926), and others. They occur in the endothelial cells of the vessels of the corium, as well as in the epidermal cells, and in the endothelial phagocytes which invade the epidermis. They are similar in every way to the nuclear inclusion bodies met with in lesions produced by the viruses of herpes, zoster and Virus III.

Ætiology.

The inability to demonstrate any formed incitant in varicella material, the history of the varicella lesion and the possibility of transmitting the condition in man with bacteriologically sterile vesicle fluid impose the conclusion that this disease is caused by an ultramicroscopic organism.

Transmission Experiments.

In man. Varicella has been transmitted to normal children by the cutaneous inoculation of fresh vesicle fluid (Kling, 1913, 1915; Lapidus, 1916–17; Gyr, 1916–17; Meyer, 1918; Hotzen, 1918–19; Greenthal, 1926). These experiments have had as their object the evolution of a method of prophylactic inoculation. A considerable percentage of children failed to react, some developed a local reaction only, others a local reaction followed by a mild attack of chicken-pox. The percentage of success has varied considerably from worker to worker, and though this may possibly be due to differences in technique, variations in the potency of the vesicle fluid and the use of the cutaneous route for inoculation are probably important factors.

Other animals. Park (1902) and Tyzzer (1905–6) failed to infect monkeys, but more recently Rivers (1926) has produced lesions characterized by nuclear inclusion bodies in the testicles of vervet monkeys. These animals were inoculated intratesticularly with emulsified chicken-pox lesions. Salmon (1905), Tyzzer (1905–6), and Teissier, Gastinel and Reilly (1922) noted no specific change in rabbits inoculated on the cornea with vesicle fluid, while Bertarelli (1909) and Gins (1918) claimed that rabbits so inoculated developed a specific keratitis. Swellengrebel (1911) and Simon and Scott (1924) obtained somewhat similar results, but doubted their specificity.

Immunity.

Active. Clinical experience points to a solid and durable immunity resulting from an attack of chicken-pox. Attempts to vaccinate children by cutaneous inoculation with fresh vesicle fluid have given conflicting results, though the work of Kling (1915) and Greenthal (1926) suggests that a certain degree of immunity can be so produced. Hess and Unger (1918) inoculated 38 children intravenously with diluted vesicle fluid (no accurate dosage given). None of the children developed any reaction, and, when later exposed to infection, one only contracted a mild chicken-pox.

Passive. Convalescents' serum has been used prophylactically and the results indicate that 4 to 5 c.cm. given during the first five days after exposure will prevent the development of the disease (Mitchell and Ravenel, 1925; Weech, 1924).

Serological reactions. Netter and Urbain (1924, 1926) state that the sera of varicella convalescents give specific complement fixation with a varicella crusts antigen, and also that the majority of these sera give equally good fixation with an antigen prepared from zoster crusts. The converse of this was also demonstrated. Lauda and Silberstern (1925) failed to confirm these findings, which, however, are in keeping with clinical observation and the cross-immunity experiments performed in children by Kundratitz (1925) (see Zoster). Valuable though the information obtained from complement-fixation reactions may be, the question of relationship between zoster and varicella will only be solved satisfactorily when a suitable experimental animal becomes available.

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CHAPTER XI. ENCEPHALITIS LETHARGICA—(EPIDEMIC ENCEPHALITIS).

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History.

ENCEPHALITIS LETHARGICA (epidemic encephalitis) was named and first described as a clinical entity by von Economo in May 1917[1] ; a few days before this, however, Cruchet, Moutier and Calmettes (1917), under the title of ' Subacute Encephalomyelitis ', published a description of 40 cases, some of which are now recognized as having been cases of encephalitis lethargica. This is evident from the clinical histories first given and from the fact that a certain number have later developed post-encephalitis symptoms (Cruchet, 1928). Cruchet's claims to priority have been fully dealt with by von Economo (1929). The bacteriology was later discussed by von Wiesner (1917).

von Economo (1917[1]), Hall (1924) and others have suggested that the disease is probably related to those indefinite historical clinical conditions, described under such names as ' nona ', ' electric chorea ' by Dubini in 1849, and ' Schlafkrankheit ' by Camarius in 1712, &c.

After 1917 the disease spread rapidly through Europe, and cases were recorded in England by Harris (1918) and by Hall (1918), and in France by Netter (1918). On account of the War the work of von Economo was then unknown in this country and the disease was investigated independently. Many of the earlier cases showed symptoms of a paralytic nature (ocular, bulbar, &c.) very suggestive of botulism. Then, as the number of cases rapidly increased, a special inquiry into the nature of this new disease was carried out under the auspices of the Local Government Board and the Medical Research Council ; the clinical and epidemiological features were studied by MacNalty and by James (1918), and the pathological side by Marinesco and by McIntosh (1918). Bacteriological and pathological examinations showed that the disease was not botulism, or a form of poliomyelitis (polio-encephalomyelitis) as had been suggested, and was, in fact, a new pathological entity. Histological examinations showed that characteristic inflammatory lesions were present in the brain, indicative of an infective process.

With regard to the relationship of the great Japanese epidemic of 1924 to encephalitis lethargica, some difference of opinion has been expressed. Certain writers, including Flexner (1927), are rather inclined to believe that they are closely related ; on the other hand, Kaneko and Aoki (1928), after a full investigation, consider that they have little in common, and that the features of the Japanese outbreak are more suggestive of the Australian X disease. As the identity of this outbreak has not been fully established, details are omitted.

Encephalitis lethargica has now been described throughout the world, although outside Europe the disease is much less severe. At the moment of writing, it seems to have become less epidemic and more sporadic in type.

Symptomatology.

The symptoms of this disease are somewhat variable, which makes the diagnosis difficult in non-epidemic periods. As observed in epidemics the signs of infection can be grouped as follows: (1) Those arising from the general intoxication; (2) Those dependent on the localization of the disease process in the central nervous system.

The onset shows considerable variation. Usually it is insidious with headache, lassitude, loss of appetite, and at times gastro-intestinal disturbances; gradually there develops the characteristic somnolence, with or without ocular paralysis. Only in a few cases, about 6 per cent., is the onset sudden with or without abrupt loss of consciousness (Parsons, 1922). The signs of general intoxication, fever, headache and gastro-intestinal disorders such as vomiting, appear mainly in the early stages.

The localization phenomena are characterized by somnolence and ocular paralysis although at times muscular rigidity and myoclonic movements may be present. In most instances the patient can be roused from his lethargy without much difficulty, and will then talk in quite a rational manner, but as soon as the extra stimulus is removed he immediately falls asleep. This clinical feature is consistent with the fact that inflammatory infiltrations and effusions in the grey matter of the base of the brain exert a very definite resistance to the passage of the ordinary external stimuli of life.

The duration of the disease is very variable and may last from 4 to 10 weeks; in fatal cases death usually occurs about the third week. Recovery is usually slow, and in a certain number of the cases manifestations of a changed mental condition, cranial nerve palsies, athetoid movements or nerve paralyses are found to persist. In others recovery seems to be complete, but is followed at a later date by symptoms consistent with chronic inflammatory changes in the central nervous system, such as give rise to Parkinson's syndrome, or impairment of mental, or perversion of moral, power. In the majority, however, recovery seems to be complete. A very full account of the symptomatology is given by Parsons (1922) in his inquiry on behalf of the Ministry of Health.

Incubation. In an insidious disease like encephalitis lethargica, not only is there difficulty in determining the actual moment at which the disease becomes manifest, but also in tracing the source of infection. Thus in only a few instances has the infection been traced from one individual to another. In the fulminating epidemic investigated by

MacNalty the incubation was short, probably only a few days. Reece (1919) gives an instance of 9 days, Kling and Liljenquist (1921) of about 10 days.

Mortality. The mortality rate shows considerable variation, thus von Economo in his second paper records 11 definite cases, of which 5 died ; while in the Local Government Board's report of 1918, 168 cases are recorded with a mortality of 22 per cent. A later report of the Ministry of Health, 1919–20, gives a mortality of 48·3 per cent. The average figures, however, work out at about 30 per cent.

Seasonal variation. Encephalitis lethargica seems to have a fairly definite seasonal prevalence, being commonest in the cooler months of the year. The number of cases increases to the end of the year, and continues relatively high during the first quarter of the succeeding year. Small outbreaks have, however, occurred at other periods.

Age incidence. Although individuals of all ages may be attacked, it was early pointed out that the incidence is greatest in young adults, and in this country about 25 per cent. of the cases occurred between the ages of 10 and 20 years, and about 20 per cent. between the ages of 20 and 30 years—the sexes seem to be equally affected.

Pathology.

Morbid anatomy. The macroscopic changes observed *post mortem* are slight, in fact the most characteristic feature is the negative nature of the examination (McIntosh). There is, however, often congestion of the cerebral vessels, and particularly those in the pons and thalamic regions, whilst the cerebrospinal fluid shows some increase. Occasionally, the grey matter of the brain is prominent, of a pink colour, and with the blood-vessels dilated. In the writer's experience hæmorrhages are not so common a feature as some writers suggest ; only two massive hæmorrhages were seen in some 20 cases ; large hæmorrhages have been recorded by Buzzard and Greenfield (1919), Bramwell and Milter (1920) ; smaller hæmorrhages are more frequent. Such appearances are consistent with the fact that most cases of encephalitis lethargica are subacute in type, and only on rare occasions are they fulminating, with definite evidence of acute inflammation with multiple hæmorrhages.

Histology. von Economo and von Wiesner, in their original papers (1917), described the microscopic appearances of five cases which came to *post mortem.* The picture, according to von Economo, was that of an acute polio-encephalitis, whose features were, especially in the acute stages, very similar to those found in acute poliomyelitis. He made no attempt to separate these two conditions. Working independently, McIntosh (1918) and Marinesco (1918) in this country, and Netter (1918) in France, described the lesions in detail and insisted that encephalitis lethargica was a pathological entity quite distinct from poliomyelitis. This raised a considerable amount of criticism at the time, as many authorities believed that this new disease was only a cerebral form of poliomyelitis.

Histologically, perhaps the most striking features of the lesions are their distribution and their perivascular character. The changes are chiefly confined to the grey matter of the brain and are most evident in the basal ganglia, midbrain, pons and upper medulla, less so in the lower medulla and much less so in the cord. The maximal effect is in the midbrain as a rule. The meninges are only slightly affected, and at most show an increase of cells here and there. Only in a few instances does the cortex of the brain show signs of involvement, while in most cases the cord is only very slightly affected, if at all. In a series of 40 cases investigated by Turnbull and McIntosh (1926) the cords were examined: at least one-third were found to be without lesions, and in no case was the involvement marked.

The chief histological lesion is an intense perivascular sheath infiltration of the blood-vessels (veins and precapillaries—Marinesco) in the areas mentioned. This cuff formation round the vessels is a most noticeable feature (Fig. 1). There are, however, in addition to this, diffuse and focal areas of infiltration in the parenchyma apparently without apparent reference to any vessel. The perivascular infiltration is, as a rule, confined within the perivascular lymphatic sheath and is composed mainly of lymphocytes with plasma and plasmoid cells (Fig. 2). Polynuclear leucocytes are conspicuously absent, although a few writers have described an increase of eosinophile cells, but this is most unusual.

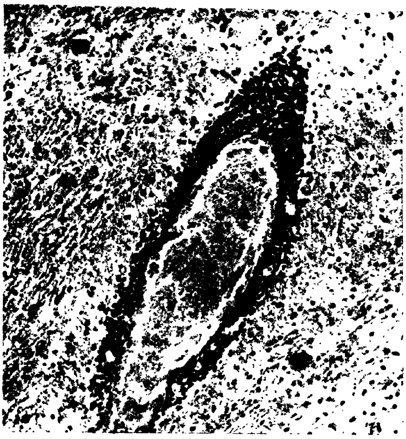
The focal areas of extra-advential infiltration are, as a general rule, diffuse, and are composed of cells similar to those found in the sheaths, with the addition of glial cells. Marinesco considers that these foci consist mainly of different types of glial cells, lymphocytes and some polynuclears.

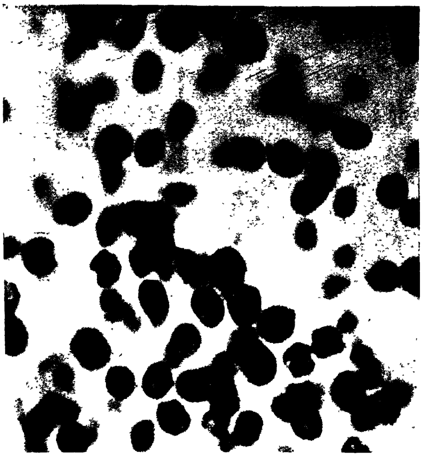
The individual ganglion cells are not much affected: they are usually well defined with the Nissl granules present. Only in severely affected localities are a few of the ganglion cells globular in shape with diffuse chromatin and some mononuclear cells in close proximity. Actual neuronophagia is rare, and in no case is such a destruction seen as to be in any way comparable to that which occurs in the grey matter of the cord, even in a very mild case of poliomyelitis.

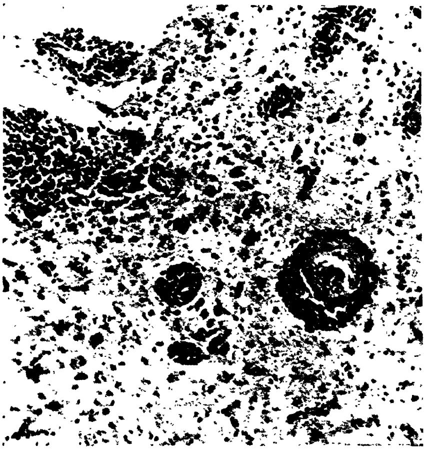
Da Fano and Ingleby (1919) and van Boeckel, Bessemans and Nelis (1923) described severe and widespread changes in the nerve-cells in isolated cases. Da Fano stated that the changes were accompanied by the presence of extranuclear granules, ‘ minute bodies ’, in the cells, which, he suggested, might be evidence of the presence of a virus, a view supported by Levaditi (1922) with his ‘ neurocorpuscules encéphalitiques ’. But it is doubtful whether the more chronic of Da Fano's cases were true encephalitis lethargica. These bodies are recognized as being distinct from the intranuclear inclusions in herpes.

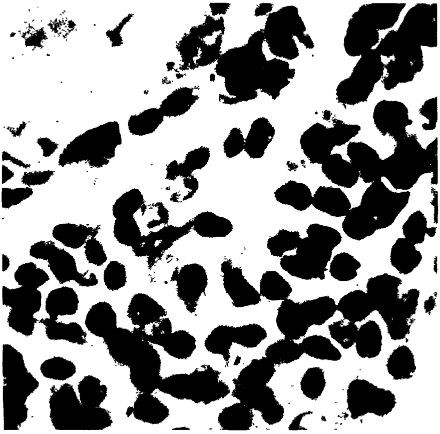
Microscopical hæmorrhages are not a general feature of the lesion, although a number of cases have been described with numerous capillary hæmorrhages (Marinesco, 1918; Netter, 1918, &c.). Change in the intima of the vessels is not usually seen and actual thrombosis is rare.

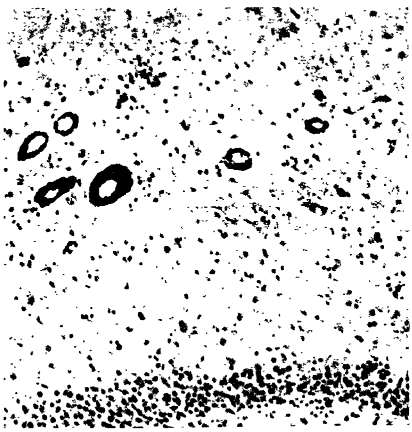
PLATE I.











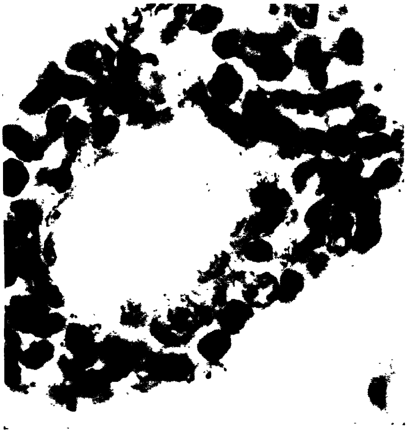
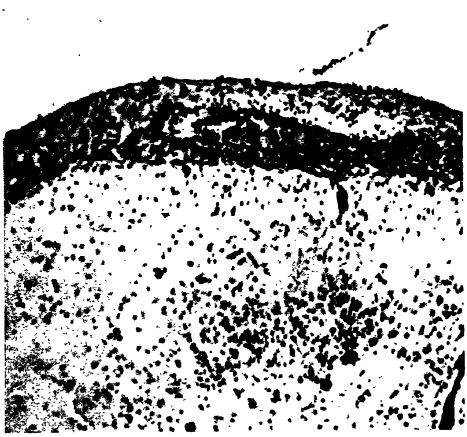
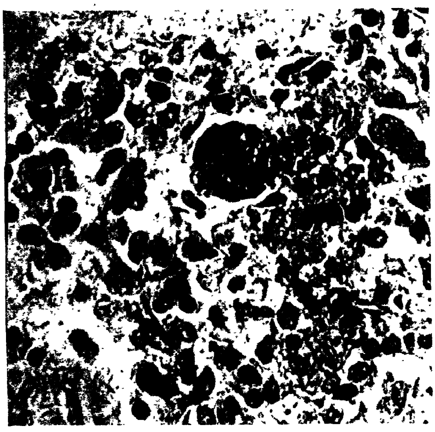
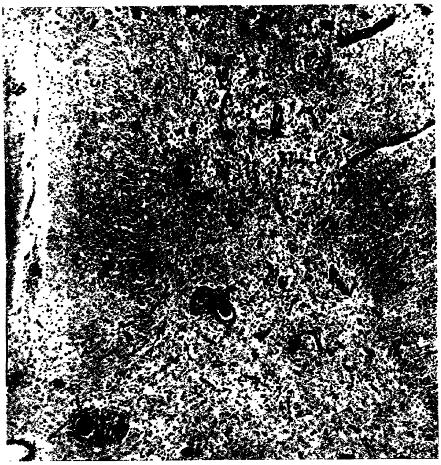


FIG. 1. Encephalitis lethargica: Section through left basal ganglia of a human case. × 100.
FIG. 2. The same. × 1,000.
FIG. 3. Experimental encephalitis lethargica: Basal ganglia of a Patas monkey. × 100.
FIG. 4. The same. × 1,000.
FIG. 5. Experimental encephalitis lethargica in a rabbit. × 100.
FIG. 6. The same. × 1,000.

[*To face p. 172.*







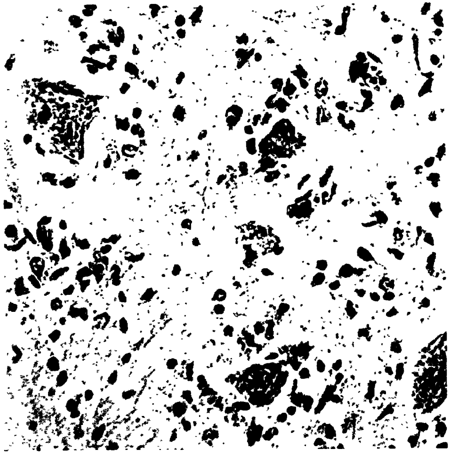


FIG. 7. Herpetic encephalitis in the rabbit. × 100.
FIG. 8. The same. × 1,000.
FIG. 9. Spontaneous encephalitis in the rabbit showing *Encephalitozoon cuniculi.* × 450.
FIG. 10. Cellular infiltration in post-vaccinal encephalitis. × 1,000.
FIG. 11. Acute anterior poliomyelitis: Cervical cord from human case. × 40.
FIG. 12. The same, showing destruction and phagocytosis of nerve-cells in the grey matter. × 760

It is generally believed that the lesions of encephalitis lethargica are slow in developing; this opinion is supported by their histological appearance. Thus, several acute cases where death has occurred within a week of the onset of the disease have shown only slight lesions in the brain (MacNalty, 1920; McIntosh and Turnbull, 1920; Lereboullet and Hutmell, 1919).

Histological changes have been looked for in the internal organs, and in the great majority of instances very little change has been found. Netter, however, stated that he found a small-cell infiltration in the parotid and submaxillary glands.

Cerebrospinal fluid. In a considerable number of instances the cerebrospinal fluid in encephalitis lethargica shows no significant departure from the normal state. Such changes as occur are usually restricted to the acute stages of the disease, and consist mainly of a slight increase of pressure, a slight pleocytosis and a small increase in the total proteid (von Economo, 1917; McIntosh, 1918; Netter, 1918; Foster, 1921; Perdrau, 1922). Perdrau states that out of a series of 269 cases in all stages of the disease, 16·3 per cent. showed a mild pleocytosis. The cells were mostly lymphocytes with an occasional plasma cell. The total protein content may occasionally show a slight increase, while the chlorides remain mostly unchanged. There is no evidence (Perdrau, 1922) of any characteristic increase in the sugar content.

Comparative Pathology.

Encephalitis lethargica, because of the type of inflammatory reaction in the brain, must be regarded as a non-purulent encephalitis, probably of virus origin. It has to be distinguished from many conditions where somewhat similar inflammatory changes are found in the central nervous system, for instance, poliomyelitis, smallpox, vaccinia, chicken-pox, measles, mumps, typhus fever and the disseminated encephalomyelitis of Redlich and others.

Cerebral poliomyelitis. Many of the original workers on encephalitis lethargica were inclined to believe that the disease was really cerebral poliomyelitis, but the characteristic histology, distribution of the lesion, the slight involvement of the cord and of the nerve-cells and animal inoculations are sufficient to distinguish these two (Figs. 11 and 12). Further, the age incidence and the general rarity of cerebral poliomyelitis are additional aids to diagnosis (cf. Chapter XII).

The other cerebral conditions from which encephalitis lethargica has to be distinguished have been found mainly in some acute exanthematous diseases which apparently form a very distinct group frequently characterized by perivascular demyelination (see Chapter VIII).

Smallpox. Clinical accounts of smallpox from time to time contain references to the appearance of symptoms which suggest involvement of the central nervous system (Turnbull and McIntosh, 1926). Recently McIntosh and Scarff (1928) gave an account of the histology of a case of encephalitis which developed after an attack of mild smallpox. The main lesion was a diffuse perivascular infiltration of the tissues of the brain by large clear cells, endothelial in type (? microglia), sparse infiltration with other inflammatory cells, and areas of demyelination round the small blood-vessels. The histological picture showed a great similarity to that of post-vaccinal encephalitis.

Post-vaccinal encephalitis has been observed in recent years after vaccination (Turnbull and McIntosh, 1926; Lucksch, 1924; Bastiaanse, 1925; Ministry of Health. Report of the Committee on Vaccination, 1928).

Measles. In measles, as a rare complication there also occurs from time to time a definite encephalitis with demyelination and cellular infiltration (Wohlwill, 1928; Greenfield, 1928). A similar encephalitis has, apparently, been found in mumps and chicken-pox (Wilson and Ford, 1927).

Disseminated sclerosis. Apparently there is an acute form of disseminated sclerosis which shows a well-marked perivascular infiltration of the vessel sheaths with demyelination (Symonds, 1924). This condition has not yet been clearly distinguished from that form of diffuse encephalitis (disseminated encephalomyelitis) described by Redlich (1927), Westphal and others. But as all of these are accompanied by well-marked perivascular demyelination, they are easily distinguished from encephalitis lethargica.

Typhus. In typhus, encephalitis is a constant feature and must be regarded as part of the actual disease process (Spielmeyer, 1919; Wolbach, Todd and Palfrey, 1922). These cerebral lesions, although essentially vascular, are diffuse and accompanied by moderate sheath infiltrations, but without demyelination.

Bacteriology.

Nature of the infective agent. Von Wiesner (1917) examined bacteriologically five of von Economo's original cases, and found in each a Gram-positive diplo-streptococcus. He found that this coccus was of low virulence, as did Evans and Freeman (1926). The great majority of workers, however, have failed to confirm von Wiesner and have, in fact, noted that the tissues are often sterile. As a result of this and from analogy with other virus infections many are inclined to believe that the ætiological agent is an ultramicroscopic virus. But with regard to virus researches, there has been a great diversity in the findings, in fact, so great that some writers think that the real agent has not yet been discovered.

The more important bacteriological investigations are given in chronological order below.

In May 1919, in America, Strauss, Hirschfeld and Loewe inoculated monkeys with brain emulsions and nasal washings from cases of encephalitis, and produced an experimental encephalitis. As in von Wiesner's experiments, the incubation period was short and there were numerous hæmorrhages throughout the brain, rather suggestive of microbic infection. They also found that they could transfer the disease to rabbits. It was stated that this virus could pass through a filter (Berkefeld N), and was resistant to 50 per cent. glycerin. Later they claimed that they were able to culture it in a special medium.

In November 1919, McIntosh and Turnbull, with material from the fulminating Derby epidemic, succeeded in causing encephalitis in monkeys, which was transmitted in series to monkeys and rabbits. The first successful inoculation was obtained in a Patas monkey inoculated with emulsion of brain tissue ; a *Macacus* inoculated at the same time remained healthy. For a period of a week afterwards the Patas monkey was quite well, then on the sixth day it had a very definite convulsive fit, from which it recovered completely. No further symptoms appeared till about a month later when it developed another very severe fit, after which it continued in a drowsy condition with minor fits and tremblings for some ten days, and then died. At the necropsy nothing abnormal was found with the naked eye, apart from some congestion of the grey matter of the brain.

From this Patas monkey the infection was transmitted to other monkeys and to rabbits with the production of typical lesions (McIntosh, 1920). Only in the case of the rabbits was an acute disease produced. The disease of the monkeys lasted for several weeks as a rule, with periods of tremblings, somnolence and convulsive movements. In the rabbit the disease became manifest in 3 to 4 days with rise of temperature, irregular movements and tremblings, while later there was definite inco-ordination and paresis, while just before death the animal was sometimes completely paralysed.

The virus was found to be capable of passing through a Berkefeld filter (impervious to *B. prodigiosus*) and showed resistance to low temperatures (—10° C.), while it could be preserved in 33·3 per cent. glycerin for several months in the ice-chest. It produced no keratitis by scarification on a rabbit's cornea. The virus was pathogenic for rabbits but only slightly so for monkeys. Several strains were raised from brain tissue, cerebrospinal fluid or nasopharyngeal washings of cases (McIntosh, 1923), but unfortunately the passage virus died out before cross-immunity experiments were made.

In February 1920, Levaditi and Harvier succeeded after a long series of failures in producing an encephalitis in rabbits with material from a human case ; after a series of passages through the rabbit, the disease was finally transmitted to monkeys, although its pathogenicity for them was slight. After a series of passages in the rabbit this virus became fixed and regularly killed the rabbits in 5 to 7 days after an intracerebral inoculation, and in 8 to 12 by corneal inoculation. Clinically the rabbits showed a rapid rise of temperature, excitement, choreic or epileptiform movements, collapse and death.

Levaditi and his co-worker ultimately raised a number of strains of virus, but most of their experimental work was carried out with two, namely C and Ch. Again, it is interesting to note the origin of these two strains; virus C was derived from the grey matter of the brain of a patient with encephalitis lethargica who died ten days after the onset of the illness, but who on admission to hospital showed a well-marked herpetic eruption. The other virus (Ch) was obtained from the nasopharyngeal secretion of a patient who recovered. Levaditi and his co-workers, therefore, obtained their viruses in both instances from material which was likely to be contaminated with herpes virus (their herpetic viruses were called N and B).

After a series of experiments on viruses C and Ch, they stated that their encephalitis virus filters readily through Chamberland filters No. 1 and 3, can be preserved in glycerin and can resist drying and autolysis. It is, however, readily destroyed by such mild antiseptics as carbolic acid or potassium permanganate; heat at 55° C. and bile have also lethal effects. This encephalitis virus was pathogenic for the rabbit, not only by the intracerebral route, but also by corneal, testicular, intraneural skin and peritoneal routes, &c. The virus was, therefore, neurotropic and dermotropic. Further experiments showed that their encephalitic and herpes viruses were identical.

Ottolenghi, D'Antona and Tonietti (1920) claimed that they were able to transmit the virus of encephalitis lethargica to guinea-pigs, but were unable to carry it on in series. In the animals which died the histological changes, however, were not like those found in the human disease. Levaditi (1922) considered that their virus was probably the spontaneous guinea-pig disease described by Jonesco-Mihaïesti.

In 1921, Doerr and Schnabel isolated a filter-passing virus from the cerebrospinal fluid of a patient with encephalitis lethargica, which, however, had only a very slight pathogenicity. They showed that the virus was in most respects very similar to that of Levaditi and Harvier. Similar viruses were obtained from the saliva of cases of encephalitis. About the same time, Netter, Cesari and Durand (1921) obtained a filter-passing virus, pathogenic for rabbits, from the brain of a chronic case of encephalitis lethargica. Apart from the occurrence of some changes in the lymphatic glands, the latter virus seems to be of the same type as that of Levaditi and Harvier.

In this country, Perdrau (1922) made several attempts to raise a virus from cases of encephalitis, but without success. As Perdrau remarks, however, his experiments did not begin until the epidemic had declined. Similar negative findings were recorded by Dible (1925) and by Douglas (1926).

In Sweden, Kling, Davide and Liljenquist (1921) successfully inoculated rabbits with material from cases of encephalitis (brain, nasopharyngeal washings, intestinal contents, &c.). The experimental disease showed great variation in the incubation period and in the symptoms. As a rule, the development of the disease was very slow, taking as a rule several weeks and at times several months. In spite of these great deviations from the acute disease produced by other investigators, Kling and his collaborators stated that the histological picture produced was identical with that found in man. An analysis of their descriptions and figures does not support their view. Whatever was the nature of their original virus, we now know that the infection which they transmitted in series in rabbits was the spontaneous encephalitis of rabbits (Levaditi, 1922). The experimental researches of Thalhimer (1921) give a very similar impression to those of the above workers in that sufficient care had not been taken to exclude spontaneous encephalitis.

Flexner and Amoss (1925), in America, made numerous attempts to raise a virus from material derived from cases of encephalitis, but without success. Ultimately, a strain of virus capable of producing encephalitis in the rabbit was obtained from the cerebrospinal fluid of a case of neurosyphilis. This virus, however, was found to have much in common with herpes febrilis (Flexner, 1927).

Viruses of the Japanese epidemic (see also p. 217). From the great Japanese epidemic of 1924, at least three different types of virus were isolated : those of Kobayashi, Nishibe and Takaki. That of Kobayashi (1925) has been shown by Cowdry (1927) to have definitely been rabies. So it must be regarded as an accident, as must the virus of Paltauf and Koritschoner, shown to be rabies by Nicolau (1925) and by Doerr and Zdansky (1924). Nishibe's (1925) results suggest that his virus was a strain of herpes. The virus of Takaki is more difficult to identify as his histological details are meagre, but it does not seem to bear any affinity to herpes, and so it may prove to be the virus of the epidemic.

Immunity.

A number of attempts have been made to demonstrate viricidal powers in the sera of convalescents and immunized animals, without any very constant results. In some instances even normal sera were found inhibitory, while in others the convalescent serum had no effect at all, or even seemed to enhance the infectivity (Levaditi, Flexner, Takaki). Thus, the effect is not comparable to what takes place in the case of poliomyelitis, while encephalitis sera have no effect on poliomyelitis virus (Amoss, 1921). Most of the immunity experiments, however, have been carried out with the idea of determining the relation of the particular virus to herpes.

Histology of Experimental Encephalitis Lethargica.

With regard to the histology of their experimental disease, Strauss, Hirschfeld and Loewe (1919) give somewhat slender details. Certainly the histological changes which they describe in the brains of the monkeys and rabbits inoculated by them do not bear any great resemblance to those found in the human disease. The intense congestion and the numerous hæmorrhages are more suggestive of an infective process of bacterial origin.

McIntosh and Turnbull (1920) give in detail the histological lesions in their experimental animals. As a rule, no pathological change of any significance was found outside the central nervous system. In the first monkey, the vessels in the meninges were slightly congested, and around some of the vessels in the pia-arachnoid there was a very slight infiltration with lymphocytes; this, however, was somewhat irregular. Within the central nervous system the most active inflammation was found in the grey matter at the base of the brain. The changes consisted of an intense perivascular sheath infiltration of the blood-vessels often forming a wide ring round the vessels. The majority of the infiltrating cells lay inside the vessel sheath, although, at times, a good number were outside. The sheath infiltration consisted mainly of lymphocytes with a good number of plasma and plasmoid cells. Polynuclear leucocytes were conspicuously few, and none were found in the sheath infiltration. In the extra-adventitial infiltration a good number of glial cells were present in addition to the cells mentioned above. Focal infiltrations were also to be seen here and there in the parenchyma, apparently without reference to any vessel. Many other changes were noticed—engorgement, hyaline thrombosis and hæmorrhages into some of the perivascular canals (Plate I, Figs. 3 and 4).

Some evidence of degeneration of the nerve-cells was observed, most of which but not all appeared to be due to post-mortem changes. Cellular changes, however, were slight. Occasional degeneration of the ganglion cells in the cord were seen. Similar lesions were found by McIntosh in a later series of monkeys, although the lesions were less intense.

In rabbits, the meninges as a rule showed a slight lymphocytic infiltration. In the cortex of the fore- and midbrain there was a variable perivascular sheath infiltration, sometimes slight, at other times pronounced. In the basal ganglia, however, it was usually distinct. The infiltration was definitely of the sheath type and the cells were mainly lymphocytes. Focal infiltrations in the parenchyma of the brain with lymphocytes and some glial cells were also to be found, although relatively rare. Involvement of the nerve-cells was slight and the majority seemed healthy. The cord, as a rule, showed no change (Figs. 5 and 6).

According to Levaditi and his collaborators, the histological lesions in the experimental disease (rabbits) is confined to the nervous system. The meninges, both on the cortex and base of the brain, usually show an intense infiltration with mononuclear cells—lymphocytes, large macrophages and plasma cells; lymphocytes predominate, but there are also some polynuclears. Their disposition is mainly perivascular. Evidence of hæmorrhages is also to be made out. Similar perivascular cuffing is well marked in the brain vessels. Inside the vessels, however, thrombi constituted of polynuclear leucocytes can be seen. These cuffs show a good number of polynuclear leucocytes, many with oxyphile granules in the infiltration (Fig. 24, Levaditi—Ectodermoses neurotropes), certainly more than are ever found in the human disease. The parenchymatous lesions are most pronounced at the base of the brain in the hippocampus region. The lesions there are such as to signify that the virus has a very selective action on the nerve-cells. The nerve-cells show the usual signs of degeneration, vacuolation, fragmentation and displacement of the nucleus and disappearance of the granules. With considerable rapidity, polynuclear leucocytes accumulate round these injured nerve-cells, and, at times, invade the protoplasm of the cells; these polynuclear leucocytes themselves often show considerable degenerative changes, such as pyknosis and fragmentation of the nucleus, and at times show acid-retaining granules (pseudo-eosinophiles), while in places neuronophagia is well marked. Levaditi has described these areas as 'véritable abcès miliaires', and in these neuronophagia is very well marked. To the interpretation of this picture the writer will return later. According to Levaditi, similar lesions were found in the monkey, but somewhat less pronounced, and meningeal hæmorrhages were present. In the guinea-pig the lesions were also very similar in type to those of the rabbit.

Kling, Davide and Liljenquist (1921) describe in the rabbit lesions consisting of a somewhat limited meningitis with infiltration of the vessel sheath. The infiltration was mainly of lymphocytes and large mononuclears. No polynuclear leucocytes were present. The cortex of the brain, as a rule, showed little or no change, but the midbrain showed the maximal lesion. Here the meningitis was well marked, and most striking were the local collections of inflammatory cells and the sheath infiltration. The infiltrations consisted of lymphocytes, polyblasts and a few plasma cells. These lesions we now recognize as being strikingly similar to those found in spontaneous encephalitis. In the more chronic form of the infection very distinct evidence of degeneration and reaction were to be seen in the local areas. Somewhat similar changes were described by Thalhimer (1921) in the brains of his experimental animals as the result of intracerebral inoculations of material from cases of encephalitis.

The experimental histological changes described by Nishibe (1925) and Takaki (1926) in their investigations on the Japanese epidemic of encephalitis are meagre and do not give enough data to enable us to form an opinion of their true nature. As already mentioned, however, there is good reason for the belief that the epidemic was not encephalitis lethargica.

Interpretation of Experimental Encephalitis in the Rabbit.

Much of the present-day confusion that exists concerning the nature of encephalitis lethargica has arisen from the fact that sufficient care has not been taken to avoid a number of pitfalls liable to be met with in experimental rabbit encephalitis. In the first place, it is important to remember that the extreme sensitivity of the rabbit to intracerebral inoculations of infected material has both its advantages and disadvantages; for by this procedure a non-pathogenic micro-organism can be made to appear virulent. Secondly, rabbits may suffer from spontaneous encephalitis, and, lastly, there must be a proper appreciation of the fact that, the experimental disease must show an almost identical histology to the natural disease.

Spontaneous encephalitis. Spontaneous encephalitis is now known to occur in rabbits in certain countries (Bull, 1917; McCartney, 1924; Twort and Archer, 1923; Levaditi and Nicolau, 1923). Histologically the lesion is distinct in that it is composed of large clear cells and definitely granulomatous in type, while in addition Levaditi (1924, 1928) and his co-workers have shown that a parasite can at times be demonstrated (see Plate II, Fig. 10).

Herpes febrilis encephalitis. The virus of herpes is now known to be capable of producing a very characteristic form of encephalitis in the rabbit by intracerebral or intracorneal inoculation (Doerr and Vochting, 1920; Levaditi, 1922). An intracerebral inoculation, as a general rule, leads to an acute disease, characterized by a high temperature—105° F. or more—tremblings, myoclonic and convulsive movements, death taking place between the fifth and eighth day on the average. The writer finds that the histological picture can be distinguished from that of true encephalitis lethargica by the severe meningitis, slight sheath infiltration, marked destruction of the nerve-cells, and the fact that the infiltration cells are different (many endothelial cells and eosinophile leucocytes, present in addition to lymphocytes) (Figs. 7 and 8), whilst in addition the characteristic intranuclear inclusions occur in the nerve-cells (Lipschütz, 1921; Goodpasture and Teague, 1923), which are quite distinct from the extranuclear bodies of encephalitis lethargica.

Vaccinal encephalitis. A vaccinal encephalitis can be produced in the rabbit by intracerebral inoculation of vaccinia virus, and the disease can be transmitted in series (Marie, 1920; Levaditi and Nicolau, 1923, and others). The inoculated animals show a very definite rise of temperature, become restless, refuse to eat and frequently show paresis. According to McIntosh and Scarff (1928) there is a very characteristic diffuse perivascular infiltration in the brain, with meningitis and at times well-marked areas of necrosis of the tissues. The infiltrating cells, for the most part, consist of cells with large, clear nuclei (endothelial type), occasional lymphocytes and glial cells. The actual nerve-cells do not show any definite change.

Relationship of the Virus of Herpes Febrilis to the Ætiology of Encephalitis Lethargica.

The position with regard to the encephalitis-herpes problem can be summed up with ease now, although it has given rise to considerable discussion. The development of the subject is interesting; apparently the idea that herpes and encephalitis were related originated in a statement by Blanc, that he saw a great similarity between certain of the so-called encephalitic viruses and the virus of herpes (Levaditi, 1926). Then Doerr and Schnabel (1921[1]) and Levaditi, Harvier and Nicolau (1922) after a series of experiments went so far as to state definitely that the virus of encephalitis lethargica and that of herpes were one and the same.

These workers apparently overlooked several important facts which are antagonistic to their view. Chief amongst these are :

1. The lesions of experimental herpes encephalitis bear little or no resemblance to those of encephalitis lethargica.
2. The intranuclear inclusions of Lipschütz, found in the nerve-cells in experimental herpes, are not present in the nerve-cells in encephalitis lethargica.
3. Herpes febrilis is an uncommon complication of encephalitis lethargica.
4. Herpes virus has only on rare occasions been isolated from nervous tissues in actual cases of acute encephalitis, and certainly with no greater frequence than it can be in other diseases of the nervous system.

Further, it has been shown by Flexner and Amoss (1925) and by Flexner (1927) that herpes-encephalitic viruses from cases of encephalitis lethargica have no greater neurotropic action than those isolated directly from cases of herpes. Later work (1928) led Flexner to consider it improbable that simple herpes and epidemic encephalitis can have a common ætiology, a view which the writer also holds.

Summary.

The discordant results recorded in the foregoing investigations make it hardly possible to give a very definite answer as regards the true nature of the infecting agent in encephalitis lethargica. The researches, however, have given us some very definite information ; the more important of this is :

1. Bacteria and, in particular, streptococci do not play any part in the ætiology of encephalitis lethargica.
2. Histological, cultural and experimental data indicate that the causal agent in all probability belongs to the filter-passing group of micro-organisms.
3. Laboratory animals are, as a general rule, very resistant to inoculation with encephalitic materials.
4. Rabbits occasionally suffer from a spontaneous encephalitis.
5. Encephalitis lethargica and herpes are distinct and separate infections.

In excuse for the fact that these findings are not more definite, it can be urged that, owing to the indefinite character of the clinical syndrome, diagnosis is difficult except during the height of the epidemic. Thus, material from a number of different affections of the central nervous system must have been examined as well as those from the real disease.

The general insusceptibility of laboratory animals to an inoculation with material from cases of encephalitis lethargica has been remarked on by most workers on the subject. It is difficult to explain why some should have occasional successes and others an unbroken series of failures.

Perhaps it is only the most active viruses that are capable of producing an infection and these are relatively rare. The writer's own work supports this view, as it was only from the most acute cases that virus was obtained, and as the epidemic died down failure resulted.

Certainly, the occurrence of spontaneous encephalitis in rabbits has confused the issue in the case of certain workers. But others again (McIntosh, Levaditi and Harvier) have clearly shown that they were not dealing with the spontaneous disease of rabbits. A similar objection has been raised against the value of positive inoculation in monkeys, based on the fact that on rare occasions these animals may show slight perivascular infiltrative lesions in the brain (Lucke, 1923). The writer feels that this objection is not valid, as he has examined the brains of a large number of healthy monkeys and never seen changes comparable to encephalitis apart from inoculation.

Again, the isolation of the virus of herpes from a few cases of epidemic encephalitis has certainly confused the issue, as it has led Levaditi, Doerr and others to consider that herpes virus is the cause of the condition. For, according to Levaditi, the viruses of encephalitis lethargica and herpes febrilis only differ in the fact that the encephalitic strain of virus is more neurotropic than dermotropic, a contention largely demolished by the work of Flexner and Amoss (1925) and Flexner (1927).

Lastly, it would appear that the inoculation of material from cases of encephalitis lethargica has given rise in animals to at least three distinct types (excluding rabies) of experimental encephalitis : (1) a hæmorrhagic encephalitis (von Wiesner and Strauss, Hirschfeld and Loewe) ; (2) the herpetic encephalitis of Levaditi and Harvier, Doerr and Schnabel, Lüger and Lauda, &c. ; (3) that produced by McIntosh and Turnbull, consisting of a non-purulent, cellular encephalitis with infiltration of the perivascular sheaths mainly consisting of lymphocytes and plasma cells, identical with the human histology and producing no keratitis.

Obviously, all three cannot be the cause, as an epidemic disease, however widespread, must have a single cause. Further work is therefore necessary to establish the nature of the infecting agent.

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CHAPTER XII. ACUTE ANTERIOR POLIOMYELITIS.

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History.

SINCE the researches of Landsteiner and Popper in 1909, acute anterior poliomyelitis or infantile paralysis has been accepted as a virus infection of the central nervous system. For the first clear clinical accounts of the disease we are indebted to Underwood (1784). v. Heine (1840) recognized the condition as a disease of the spinal cord and Cornil (1864) of the grey matter of the cord. Charcot considered it to be the result of a primary degeneration of the anterior horn-cells, a view which has been subject to much discussion.

The exact nature of the pathology of the disease was finally established by the work of Harbitz and Scheel (1907) and Wickman (1911), who demonstrated that it was inflammatory in character, a belief confirmed later by experimental investigations of Landsteiner, Levaditi, Flexner, Müller, Römer and others.

Poliomyelitis is usually characterized by a sudden onset, often with gastro-intestinal disturbances, fever, malaise, drowsiness and a rapidly developing flaccid paralysis of one or more limbs. Death as a rule only occurs when the respiratory muscles are affected, but the infection leaves the great disability of permanent limb paralyses. Clinically, several forms are recognized, depending on the part of the central nervous system most affected; thus, three main forms occur, namely, spino-bulbar, cerebral and abortive, with clinical variants. For a full account of the clinical and epidemiological features the reader should consult the monographs of Peabody, Draper and Dochez (1912), Batten (1916) and MacNalty (1927).

Epidemiology.

In Great Britain, poliomyelitis is mainly endemic, although epidemics of varying severity appear from time to time. In Sweden, epidemics involving many hundreds of cases have been recorded in 1887, 1895 and 1905, and smaller outbreaks in the intervening periods. In the United States, the most severe epidemics have occurred in the Eastern states, particularly in New York (1905) and Massachusetts (1909–10) and in Chicago (1915–16). It is supposed that the infection was brought to the eastern seaboard of America by immigrants from Sweden. In recent years, Canada and New Zealand have suffered severely from poliomyelitis, and particularly the latter in 1916 and again in 1924.

The incubation period varies from 5 to 14 days and is about a week on the average ; it may be as short as 3 or 4 days. Müller (1910) and Römer

(1911) place it at about a week, and an analysis of Kling and Levaditi's cases gives the same period, though these authors state that it may be as short as 3 or 4 days; the shortest period observed by Römer was 5 days.

The mode of infection, as shown by Treves (1909), Wickman (1911) and Kling and Levaditi (1913) is chiefly direct, in that the infection is mainly spread by cases and by carriers. Only in a few instances has the disease been traced to fomites. Wickman, in the great Swedish epidemic of 1905–7, owing to the isolated nature of many of the outbreaks (in islands) had particularly good facilities for determining these points. For instance, in the Parish School of Träscoma, 49 cases occurred and the infection was traced in 44 cases. In another outbreak the infection was carried to 12 homes by 12 different children, only 6 of whom ever showed any symptoms of the disease. Somewhat similar instances were given by Kling and Levaditi (1913).

Mortality. In general, the mortality apparently varies from 7 to 25 per cent., but owing to the fact that many abortive and mild cases are missed, it must in reality be considerably lower. But in the different epidemics much variation was shown in the different countries. The mortality is influenced by age, being as a rule higher as the child's age increases (Bruce Low, 1917; MacNalty, 1927).

Seasonal variation. The incidence is highest in the hottest months of the year, usually July, August and September, but the disease may occur in any month.

Age Incidence.

Acute poliomyelitis is essentially a disease of infancy; some 70 to 90 per cent. of all cases occur before the fifth year of life, and the highest incidence is in the second year (cf. MacNalty, 1927).

Changes in Blood and Cerebrospinal Fluid.

Blood changes. According to La Fétra (1909) and Peabody, Draper and Dochez (1912), the blood changes in poliomyelitis are very definite, and consist chiefly in a rise in the number of white cells. This leucocytosis on the average varies from 15,000 to 20,000 or more, with the polynuclear leucocytes increased by 15 to 20 per cent. above normal. The increase of leucocytes may be quite marked even in the pre-paralysis stage. Müller (1910), however, states that in the earliest stages there is often a leucopenia.

Cerebrospinal fluid. The changes described in the cerebrospinal fluid have not been constant. Peabody, Draper and Dochez (1912) in their monograph state that the commonest finding is a normal or slightly increased cell count with a well-marked globulin reaction (meningeal type of reaction). The highest cell counts were found in the early stages of the disease, including the preparalytic stage; thus, in the first week of the disease about 90 per cent. showed an increased cell count, by the third week this figure had dropped to less than 25 per cent. The predominating type of cell is always mononuclear (lymphocyte), although at times this cell is somewhat difficult to classify. Only in the very early stages (preparalytic) are polynuclear leucocytes present in considerable numbers, and Aycock and Luther (1928) lay stress on the value of lumbar puncture in the diagnosis of this stage. Durand (1919) gives the average cell count as 150 cells per c.mm. with lymphocytes predominating. He also states that the fluid, on withdrawal, is usually clear. The globulin content, on the other hand, rises as the disease progresses and as the white cells decrease.

Pathology.

Morbid anatomy.

Post-mortem examinations of acute cases show, as a rule, some parenchymatous degeneration of the internal organs, kidney, liver, &c., with a general enlargement of the lymphatic glands, including the lymphoid tissue of the intestinal canal. The cord is usually congested, soft and œdematous with minute hæmorrhages in the grey matter; at times it may be pinkish in colour on section.

Histology.

Briefly, the histological lesions may be summarized as follows:

Central nervous system. (1) The most pronounced lesions are to be found in the grey matter of the cord and medulla and, to a much less extent, in the grey matter of the base of the brain, whilst changes in the cortex are relatively slight. The meninges (pia mater) are also involved in the process.

(2) The inflammatory reaction is of a double character; (*a*) a marked perivascular sheath infiltration of the vessels of the grey and white matter, and (*b*) diffuse and focal infiltrations of the grey matter. This sheath infiltration involves the meningeal vessels as well; the cells in the sheath are closely packed and often form a wide cuff round the vessel. Occasionally there is some overflow of the diffuse infiltration into the white matter.

(3) The most characteristic histological change is the degeneration of the ganglion cells of the grey matter of the cord. All stages of cellular degeneration, from a slight chromatolysis with loss of Nissl granules to complete destruction with neuronophagia may be found, and not infrequently the affected cells are granular in appearance. These granules are also found outside cells and are acidophile in reaction; it has been suggested that they may actually be the parasite of poliomyelitis.

(4) Small hæmorrhages in the grey matter are a usual feature and vary in number and extent, according to the severity of the intoxication.

(5) Cytologically, a number of different cells can be determined. The sheath infiltration consists largely of lymphocytes with a small number of polynuclear leucocytes and a still smaller number of larger cells with clear nuclei. The infiltration of the grey matter is composed of similar cells, but with a larger number of polynuclears and with the addition of

glial cells. In the meninges the cellular accumulations consist mainly of lymphocytes and cells of the endothelial type. Plasma, plasmoid cells and lymphoblasts are rare (Wickman, 1911; McIntosh and Turnbull, 1913). Cortical lesions are of a similar type but more focal. The posterior root ganglia frequently show a varying degree of cellular infiltration and degeneration of the ganglion cells, and on the whole the changes are very similar to those present in the cord. Some of the nerves, according to Levaditi and Stanesco (1910), may show an inflammatory reaction as well.

Other organs. The microscopical lesions of the other organs consist of focal areas of necrosis in the liver, and an inflammatory reaction of the lymphoid tissue, including that of the intestine.

The lesions described above vary considerably according to the stage of the disease. Polynuclear leucocytes will be more numerous in the early stages. The earliest change which has been found in the central nervous system, either in the natural or experimental disease, is an infiltration of the pia-arachnoid with small mononuclear cells (Leiner and v. Wiesner, 1910). This has been described by Peabody, Draper and Dochez (1912) as an acute interstitial meningitis which is usually most marked on the anterior surface of the cord, medulla and base of brain and involves the perivascular lymph spaces. Later, hæmorrhages and œdema are constant features, while still later, sclerotic lesions are to be found.

Mechanism of the production of the cord lesion.

By whatever route the virus of poliomyelitis gains access to the tissues, it is now generally believed that it has some special affinity for the ganglion cells of the cord; their destruction depending on the direct action of the virus and not on any secondary vascular influence. Levaditi (1922) considered that the virus soon leaves the site of entrance or inoculation and immigrates to the central nervous system, where it multiplies without producing any changes for a time. This is confirmed by experimental work in monkeys, which has shown that, no matter where the virus has been introduced (brain, nerves or peritoneal cavity), the disease always begins as a localized paralysis of the limbs, and this is also supported by the fact that the virus is seldom, if ever, to be found in the blood.

Bacteriology.

For some years before Landsteiner's discovery there was a considerable diversity of opinion as to the nature of the infecting agent. One type of microbe was isolated by one group of investigators and others by other workers (Schultze, 1905; Potpeschnigg, 1909; Rosenow *et al.*, 1916). The organism most frequently isolated was a Gram-positive coccus, usually pleomorphic in character. On the other hand, other investigators insisted on the general sterility of the cerebrospinal fluid and nerve tissues in acute poliomyelitis (Perkins and Dudgeon, 1907; Wickman, 1911; Starr, 1908; Wollstein, 1908, *et. al.*).

At the present time, Rosenow alone seems to be convinced of the importance of a coccus as the ætiological agent in poliomyelitis; he claims to have prepared an antiserum for poliomyelitis with this coccus, but the researches of Amoss and Eberson (1918) have shown that Rosenow's serum has no protective or viricidal action on poliomyelitis virus. Rosenow is supported in his belief by the fact that it is relatively easy to culture streptococci from poliomyelitic tissue from men or monkeys who have died of the infection. These are, however, usually classed as contaminants or agonal infections (Fairbrother, 1929).

Comparative Pathology.

There are a number of acute infections of the central nervous system which have never been clearly differentiated from poliomyelitis, e.g. Landry's paralysis, amyotrophic spinal paralysis (Werdnig-Hoffman), polio-encephalomyelitis (Strümpell), Australian X disease, Borna disease of cattle (enzootic encephalomyelitis). Some of these show a very close similarity, and in fact may only be clinical varieties rather than pathological entities.

Although the exact pathology of the ascending paralysis described by Landry is not very clear, it is now generally agreed that many of the cases are merely ascending forms of poliomyelitis. Amyotrophic spinal paralysis (progressive spinal muscular atrophy) is a rare condition: only some 20 or 30 cases are on record. From one of these, Landsteiner, Levaditi, Prazek and Zalelli claim to have isolated the virus of poliomyelitis. The polio-encephalomyelitic group of Strümpell seems to have been inadequately investigated, though it is probable that a number of his cases were merely types of cerebral poliomyelitis.

The Australian X disease first appeared in Australia in 1917–18. This encephalomyelitis was characterized by a very high mortality (70 per cent.), but paralyses were only present in about 10 per cent. of the cases. Widespread inflammatory changes were present in the cord with sheath infiltration and destruction of the nerve elements. The relative rarity of paralysis, the greater mortality and the fact that the disease could be transmitted to horses and sheep as well as to monkeys, suggest that it was due to an unknown virus and was not an acute form of poliomyelitis.

Borna disease, as far as is known, only affects animals, particularly horses, sheep and cattle; the histological lesion, however, shows a great similarity to that of acute poliomyelitis (McIntosh and Scarff, 1928), while recent experimental work of Nicolau and Galloway (1928) indicates that a certain degree of cross immunity exists between this disease and poliomyelitis.

Borna disease is described in detail elsewhere (Chapter XXVIII).

Nature of the Virus.

Transmissibility.

The infective nature of acute anterior poliomyelitis was proved by Landsteiner and Popper's (1909) discovery that it could be transmitted to monkeys. Not only was this work soon confirmed, but it was shown that the disease could be transmitted in series in monkeys (Knöpfelmacher, 1909; Flexner and Lewis, 1909; Leiner and v. Wiesner, 1909; Landsteiner and Levaditi, 1909; Römer, 1909). At first inoculations were all made intraperitoneally, but later intracerebral inoculations were found to be the most constant; according to Leiner and Wiesner the order of infectivity of the various routes is: intracerebral, intraneural, intranasal, intraperitoneal, subcutaneous, intravenous.

Landsteiner and Popper inoculated two monkeys (*Macacus rhesus* and *Cynocephalus hamadryas*) with fragments of cord emulsified in saline. The first monkey developed paralysis on the sixth day and died on the eighth, the other became paralysed on the seventeenth day. Inoculation in series failed. These authors showed that the lesions obtained were histologically identical with those found in the natural disease. The experimental disease in the monkey shows a very close resemblance to poliomyelitis in children. There is an incubation period of from 4 to 17 days, as judged from the protocols of Römer and of Levaditi, but on the average it is about 6 to 10 days; Flexner and Lewis state that it may be prolonged to 27 or 33 days. Any signs of infection previous to the development of paralysis are very slight and usually not noticeable. Just before the onset of paralysis there is a rise of temperature, loss of appetite, tremblings and increased timidity. The paralyses usually appear within 24 to 48 hours of the rise of temperature and affect the lower limbs first and then ascend till a condition of general paresis ensues, which is quickly followed by death. The disease more often causes death in these animals than in the human subject. When the attack is less severe the affected limb or limbs are found to be quite flaccid, but after a time some of the power may be recovered, or atrophy of the muscles follows. Occasionally involvement of the cranial nerve-centres may occur. The disease is sometimes very mild and the animal recovers completely in a few days.

The post-mortem appearances are similar to those found in man; there is congestion of the cerebral and spinal meninges, while the grey matter of the cord may be pink and contain a few petechial hæmorrhages. Histologically the changes are identical with those found in man; a very complete study of these changes is to be found in a recent publication by Hurst (1929).

Method of obtaining the virus.

In order to obtain the poliomyelitis virus from a human case, the cord should be removed with all aseptic precautions as soon after death as possible (the virus is present throughout the nervous system, but is present in greatest amount in the cord) and placed in 33⅓ per cent. glycerin in a stoppered bottle. For inoculation a small section of the cord is taken and rubbed up in a small mortar with normal saline so as to make a 10 to 20 per cent. emulsion. As the cord is usually very soft, it is unnecessary to use sand. The gross particles are allowed to settle and the top layer is then taken up in a syringe. From 1 to 2 c.cm. of this is injected directly into the cerebrum of an anæsthetized monkey. In order to study the progress of the infection the monkey's temperature should be taken daily. As soon as any symptoms are manifest it is best to kill the animal and make a second passage, as a rapid recovery is apt to occur and the virus be lost.

Animals receptive.

Monkeys can be infected in about 90 per cent. with a virulent passage virus, with a mortality of about 75 per cent., and old world monkeys seem to be more susceptible than new world (Flexner and Lewis, 1910). With an initial passage from man to monkey the infectivity is much lower, 50 to 60 per cent., and the mortality 20 to 40 per cent. Rabbits have only on rare occasions been infected (Krause and Meinicke, 1909; Dahm, 1909; Marks, 1911). Landsteiner and Levaditi obtained only one positive result out of a large number of inoculations, while completely negative results were obtained by Flexner and Lewis (1910), Römer (1911), Leiner and v. Wiesner (1910). Other animals, such as the horse, ox, pig, sheep, dog, cat, rats and guinea-pig have been found to be quite resistant (Flexner and Lewis, 1910; Landsteiner and Levaditi, 1910; Leiner and v. Wiesner, 1910).

Distribution of the virus.

The investigations of Landsteiner and Levaditi (1909), Flexner and Lewis (1909) and others have all shown that the virus of poliomyelitis has a special affinity for the central nervous system and in particular for the grey matter of the cord. The blood rarely contains it, while the internal organs, spleen, liver, kidney, &c., are free.

In experimental poliomyelitis Flexner and Lewis (1910) found that the virus is also present in the nasal secretion, nasal mucosa, tonsils and other lymphoid tissues, while the cerebrospinal fluid, as is the case in the human disease, does not contain it in any stage of the disease. After intravenous injections, the virus soon disappears from the blood and is only to be found in the lymphatic tissue and bone-marrow (Flexner and Amoss, 1914); apparently some mechanism prevents it from reaching the central nervous system after intravenous injections if the membranes are intact. But, on the other hand, experiments have shown that various secretions or excretions may contain the virus, e.g. nasopharyngeal secretion, urine, fæces, &c. Kling, Wernstedt and Pettersson (1912) state that some 20 to 30 per cent. of such secretions gave positive results on inoculation. They also found that similar results were obtained both with abortive cases and with carriers. Flexner and Amoss (1914^2), however, failed to confirm their results with regard to fæces.

The above findings suggest the mechanism by which the virus of poliomyelitis gains entrance to and spreads in the body. Apparently it gains access to the tissues by the nasopharyngeal or intestinal route.

In either case it seems to migrate rapidly to the lymphoid tissues and from there progresses by the perivascular or perineural lymphatic spaces to the central nervous system. There, according to Levaditi, it can multiply for some time without producing any very evident signs.

Filterability.

Experimental inoculations show that there is a considerable amount of variation in the virulence of different strains of poliomyelitis as tested on the monkey; on the average, however, 50 to 60 per cent. of the cords from cases of poliomyelitis can be successfully inoculated into monkeys (Flexner, Römer, Levaditi, McIntosh and Turnbull). It is generally agreed that the infective agent belongs to the filter-passing group of micro-organisms, since it can pass bacteria-holding filters such as Berkefeld, Chamberland, Reichel and Nordtmeyer (Landsteiner and Levaditi, 1909; Flexner and Lewis, 1909; Strauss and Huntoon, 1910; Leiner and v. Wiesner, 1910. A proportion of the virus is, however, retained by the filter, since the incubation period is always lengthened when a filtrate is used. In the reports quoted above, the exact porosity of the filters are, as a rule, not stated, apart from the general type of candle. The filtration of the virus, however, can readily be made by making a 5 per cent. emulsion of cord in saline, filtering first through paper or sand and paper pulp, and then through the selected filter by the aid of a little suction. By this procedure the virus will be found to pass readily through the coarse Berkefeld (V), but with much greater difficulty through Berkefeld (W) or Pasteur-Chamberland No. 2.

Resistance.

The virus is endowed with considerable resistance to external (physical) agencies. It can be kept for months or years (6 to 8) in the refrigerator alone or in contact with glycerin. For conservation it is best kept in 33⅓ to 50 per cent. glycerin in an ice-chest. Flexner and Amoss (1917) kept a sample for 6 years in 50 per cent. glycerin.

According to Landsteiner and Levaditi (1910) the virus can resist complete drying over potassium hydrate. Leiner and v. Wiesner (1910) found, however, that it was readily destroyed by heat, 20 minutes at 60° C. being quite sufficient. Cold, on the other hand, is apparently without effect, as it can be kept indefinitely at temperatures of 12° C. or lower. The action of antiseptics is somewhat variable. Considerable resistance is noticed, for instance, against 0·5 per cent. carbolic acid and against thymol, but the virus is easily destroyed by such agents as potassium permanganate, 1 in 1,000, or hydrogen peroxide, 1 per cent. While bile and bile-salts have a pronounced destructive action (Landsteiner, Levaditi and Pastia, 1911[1]), gastric and intestinal secretions are without effect (Flexner, Clark and Dochez, 1912). Normal brain-tissue seems to have no neutralizing effect on the virus and no absorption takes place (Landsteiner, Levaditi and Pastia, 1911[1]).

Cultivation.

Flexner and Lewis and Levaditi early in their experiments noted that when culture tubes containing a medium composed of nutrient broth and serum (monkey serum or ascitic fluid) were inoculated with poliomyelitic nerve-tissue and incubated at 37° C., a turbidity developed in the lower part of the tube. Stained specimens from the tubes showed numerous fine rounded particles, which, according to Levaditi, suggest a very small micro-organism, as particles similar to these are not infrequently found inside or just outside affected ganglion cells in the spinal canal. Later, Flexner and Noguchi (1913[2]) restudied this question and as a result claimed to have definitely cultivated the virus of poliomyelitis on the medium devised by Noguchi for the cultivation of spirochætes. Later, Flexner, Noguchi and Amoss (1915) claimed to have reproduced poliomyelitis with a strain of the virus which had been cultivated for a year and a half. An examination of the culture tubes showed that the cloudiness was due to the presence of minute rounded bodies or 'globoid bodies' as they called them. Somewhat similar bodies were found by Römer with the ultramicroscope and by Noguchi in the poliomyelitic tissues. Amoss (1917), however, could not determine any immunological relationship between the bodies and the virus itself, nor have these bodies been found in highly virulent filtrates. Although Amoss (1917), Smillie (1918), Heist, Solis-Cohen and Kolmer (1918) claim to have obtained similar cultures, the actual value of these results is somewhat doubtful.

Immunity.

That some degree of immunity, both natural and acquired, exists against acute poliomyelitis is evident on clinical grounds. A second attack is almost unknown and only a small proportion of the children (less than 5 per cent., Wickmann) exposed to infection develops the disease. In experimental work also it is found that monkeys which have recovered from an attack are immune to a second inoculation. The production of an active immunity in monkeys by vaccination was attempted, but without success, by Landsteiner and Levaditi (1910), Flexner and Clark (1911), Römer and Joseph (1910), and others, with attenuated and heated virus. Krause (1911) claimed positive results with carbolized virus. The mild virus of Flexner and Amoss (1924), however, produced a very definite immunity. A reinvestigation of this subject has enabled Stewart and Rhoads (1929) to conclude that it is impossible to protect monkeys with killed virus, but that a definite though inconsistent resistance can be produced by subcutaneous inoculation or, better still, by intradermal introduction of the living virus.

Early in their experimental researches in poliomyelitis, Flexner and Lewis and Landsteiner and Levaditi found that the serum of recovered monkeys contained protective substances against the virus, while Römer and Joseph, Netter and Levaditi found similar bodies in the serum of recovered human cases. The actual experiments showed that these sera had neutralizing action on the virus both when tested *in vivo* or *in vitro*. Serum from convalescent cases of encephalitis lethargica has no such viricidal power (Amoss, Levaditi, McIntosh—unpublished). Similarly, poliomyelitic serum has no action on rabies virus (Landsteiner and Levaditi, 1910). The amount of poliomyelitis antibody present is small and the virus must be left in contact with the serum for several hours to ensure complete destruction. Flexner and Lewis (1910) found that the serum could prevent the disease in monkeys if injected some time before the virus. The complement-fixation test so far has failed to show the presence of antibodies in convalescent serum (Römer and Joseph, 1910; Gay and Lucas, 1910).

Propagation of the Disease.

Infection is in most instances direct from those suffering from the disease or from abortive cases or carriers. Wickmann in his clinical study was the first to bring forward evidence that the infection could be carried by food (particularly milk), books, drawings or other intermediaries. Landsteiner and Levaditi found that the virus could remain alive for 30 days in milk at room temperature. Aycock (1927) and Rosenau (1928) have noted several instances where there is good evidence for believing that the infection was carried by milk. Landsteiner, Levaditi and Pastia (1911^2), Flexner and Amoss (1920), and Kling, Wernstedt and Pettersson (1911) have shown that infection is spread mainly by virus present in the nasopharyngeal secretions and saliva. Absorption can apparently take place through the normal mucous membrane, although irritation facilitates the passage. From thence it passes along the lymphatics accompanying the branches of the olfactory nerve to the grey matter at the base of the brain. Leiner and v. Wiesner's (1910) observation confirmed Wickmann's view that infection may occur through the intestinal tract by the ingestion of contaminated articles of food; but the fact that they used opium to paralyse the gut has been urged against the validity of their experimental finding. Moreover, Flexner, Clark and Fraser (1913), Kling, Wernstedt, Pettersson ($1912^{1\,\&\,2}$) and Osgood and Lucas (1911) found that the virus can persist in the nasal and other secretions for weeks or even for months after an attack, while it has also been found in the nasopharynx before the onset of the paralytic symptoms.

Dust may be a possible source of infection, as Levaditi showed that the virus can resist drying, but so far the only successful attempt in transmitting the disease to monkeys with dust from an infected room has been that of Neustaedter and Thro (1911).

No satisfactory evidence has been brought forward to show that any biting insects, such as the stable fly (*Stomoxys calcitrans*) as stated by Rosenau (1912), play any part in the spread of the disease [Anderson and Frost, 1912; Kling and Levaditi, 1913; Sawyer and Herms, 1913; Noguchi and Kudo (mosquitoes, &c.), 1917].

Prophylaxis.

The researches described indicate the lines along which prophylaxis may be practised in an epidemic of poliomyelitis. Means should be taken to keep the nasopharynx free from infection, by the use of gargles, nasal douches or sprays. Even such a simple remedy as normal saline can remove much of the virus. In addition, individual cases should be isolated. Protection by active immunization has not so far been attempted in man, although passive immunization with antipoliomyelitis serum has been suggested. Flexner and Stewart (1928) have advocated the injection of 5 to 10 c.cm. of convalescent serum intramuscularly as a prophylactic.

Treatment.

The rapidity with which the paralyses develop after the first manifestation of the disease makes treatment difficult. Anything which is to do good must act quickly, and, from this aspect, serum therapy, and particularly if given in the preparalytic stage, alone holds out any hope.

Convalescent serum. Following the discovery of antibodies in convalescent sera, Netter (1915) treated several cases with good results, and since then the use of the serum has been more general. Zingher (1917), Aycock and Luther (1928), Marinesco, Manicatide and S.-Draganesco (1929) have all reported favourably on its action in preventing paralyses. For instance, Aycock and Luther treated 106 cases in the preparalytic stage :

	Treated. (106)	*Untreated.* (482)
	—	—
Mortality	0·94 per cent.	14·0 per cent.
No paralysis	36·0 ,,	1·2 ,,
Severe paralysis ..	5·7 ,,	46·6 ,,

All writers seem agreed that the best results are only obtained when the treatment is commenced in the preparalytic stage, which has a duration of 48 to 72 hours. The treatment may be intraspinal or intravenous or a combination of the two. As a rule, 10 to 20 c.cm. is given by the intraspinal route and 30 to 100 c.cm. intravenously. The intraspinal injection should be repeated in 12 to 24 hours. Injections of normal serum have not been found to give good results, nor has the autohæmotherapy of Sicard, Haguenau and Wallich (1927) much to recommend it. As a prophylactic dose, Flexner has recommended 10 to 20 c.cm. by subcutaneous injection. The serum should be collected from convalescents a few weeks after recovery.

Hyperimmune serum. Attempts to prepare a hyperimmune serum from animals (horse and sheep) have, in the hands of Flexner and Lewis, Amoss and others given unsatisfactory results. Pettit (1918), on the other hand, claims a considerable amount of success, but so far the results (Etienne, 1927 *et. al.*), which have come to hand, are not very convincing.

Non-specific treatment. This mainly consists of the injection of diffusable antiseptics, such as urotropin (Flexner and Clark, 1911) or hypertonic saline (Aycock and Amoss, 1923), and has not given very hopeful results.

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CHAPTER XIII. RABIES.

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Introduction.

The causative agent of rabies belongs to the group of ' filterable viruses ', and it was one of the first members of this group to be recognized. It possesses the characteristics which pertain to filterable viruses in general. The localization of the virus is highly selective, for only nervous tissues are affected. The virus is transmitted within the body along the nerve-trunks only, and the direction of transmission is both centripetal and centrifugal. For the successful transmission of the disease by animals it is necessary that the saliva or other alimentary secretion containing the virus should be brought into contact with the nerve-tissues of another animal. The virus is then transmitted along a nerve-trunk to the central nervous system. The period of incubation varies with the distance to be travelled. In man, for implantation on the head, it is approximately 27 days, on the arm, 32 days, on the leg, 64 days, but these figures are subject to wide variations. During this period no symptoms are apparent.

Symptomatology.

Rabies is one of those diseases which attacks the lower animals and man alike. It produces very much the same train of symptoms in all animals infected. At first the animal shows altered behaviour. It seeks secluded corners in which to hide, ceases to be obedient, although there are cases in which an excess of affection may be shown, and it has a distinctly perverted appetite. The change of appetite is one of the most characteristic features of the disease in lower animals. It will gnaw at and swallow paper, sticks, straw, earth, leather and similar unusual materials. These early symptoms are not always pronounced, a fact which contributes to the difficulties of recognizing the disease at an early stage, when there may possibly be no suspicion of any infection having previously taken place.

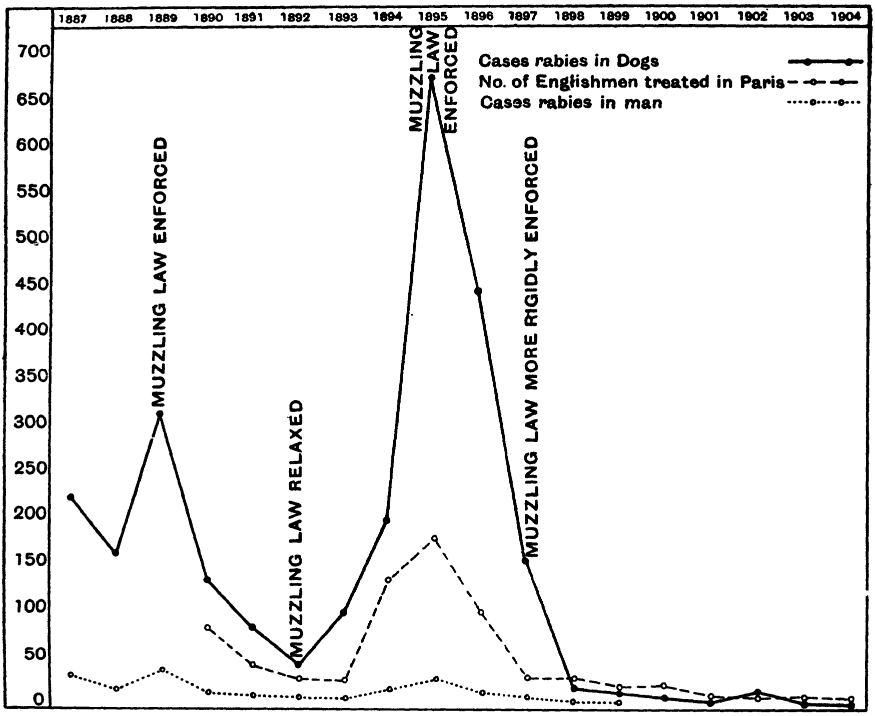
This stage is succeeded by one of excitement, in which snapping and unprovoked biting at other animals, aimless wandering, erratic and staggering mode of progression, outpouring of unswallowed saliva from the mouth, alteration of the bark to one of characteristically high-pitched tone are prominent features. This is the dreaded mad dog stage. The animal is still active and capable of dangerous aggression. Furious rabies, as it is called, may last for one or two days, and is followed by 'dumb rabies', the paralytic end phase. If the furious form is short, as it may be, the dumb form may supervene without the former ever having been observed at all. In this form a staggering gait occurs, indicative of paresis of the hinder extremities; paresis goes to definite paralysis and to involvement of the anterior extremities. The lower jaw becomes paralysed and thick ropy saliva streams from the mouth. In this state of helplessness, the animal may exhibit from time to time muscular spasms which affect the whole body. Emaciation is very marked and greater in degree than is accounted for by the inability to partake of food during the few days that the disease lasts. The termination of the disease is death, although in very exceptional cases, reports of recovery and even of recurrent rabies in animals are published. The generally accepted practice is that treatment is considered unnecessary if the animal remains in sound health for ten days after the bite was inflicted.

There is no apparent fear of drinking in the dog, such as is manifest in the human being, but the progressive inability to swallow is found equally in both. Not all lower animals affected with rabies bite indiscriminately; among those that do not are guinea-pigs, rabbits, oxen and horses. Some of these, however, and also the dog, will, in the early stages, lick or even savagely bite at the wound through which infection has entered, as if there were peripheral and localized irritation there of nerve terminals.

In man, the course of the disease is similar to that in the dog. Absence of certain manifestations such as those of biting and presence of others such as fear of drinking may be ascribed to the higher mental organization and power of restraint of man. Fear and anxiety are prominent from the very commencement in individuals who are aware of the possibilities of their condition. As the disease progresses excitability increases. Not only the attempt to quench a torturing thirst, but the thought of water or the sound of running water, will bring on pharyngeal spasm. Other external stimuli such as a draught of air, a noise, a movement, become the exciting conditions of a distressing paroxysm. Respiration becomes laboured, exaggerated and noisy with held respiration, and there develops the state of aerophobia. Generally speaking, there is no attempt to bite, such as is characteristic of the dog, the cat, the wolf, and the jackal. Consciousness remains practically to the end. Paralytic manifestations supervene upon the state of increased excitability and this is the regular order of events in man. Death occurs in 3 or 4 days from onset either quietly or in the midst of a paroxysm.

Epidemiological Peculiarities.

The disease may occur in any country and in every climate. No seasonal variations are observed. The relative incidence in different countries depends upon geographical conditions. In the case of countries which are isolated from their neighbours (e.g. islands, peninsulas, &c.), methods of eradication have been successful. The following chart shows how the muzzling of dogs succeeded in stamping out rabies from Great Britain:



In a similar manner the disease has been eradicated from the Scandinavian countries, Norway, Sweden and Denmark, and from Holland. It has apparently never gained a footing in Australia.

Although many classes of vertebrata may be affected, the disease may, for practical purposes, be considered as primarily a canine enzootic, and its occurrence in man or other animals as merely incidental. Consequently, quarantine regulations against the dog alone have proved successful where frontier conditions have been such that control of this nature could be enforced, thus indicating that the effects of transmission of the disease by other susceptible animals is negligible. The innocuousness of these animals may depend upon the fact that many do not bite deeply, or upon non-infectivity of their saliva. Ouchakoff reports only one death from rabies amongst 2,859 persons afforded antirabic treatment in the Russian Institutes who were bitten by horses, and no deaths amongst 3,522 who were bitten by rabid cattle. At Kasauli in India, out of 22,519 patients who required antirabic treatment, 80 per cent. did so owing to contact with dogs, 18 per cent. with jackals, and 0·3 per cent. with cats and wolves (McKendrick and Fox, 1917).

The disease is prevalent under natural conditions as an enzootic. Epizootics are very seldom reported. This is to be expected both from the wideness of the variation of possible incubation periods and also from the fact that transmission appears to be independent of climatic and seasonal variations. Whilst all members of the canine tribe may be involved, viz., dog, wolf, jackal, fox, &c., and whilst these rabid animals may travel far during the period of their infectiveness (Corwin, 1924: average distance, 18 miles; greatest distance, 40 miles), it would appear that local measures against the dog are in the main very successful.

Animals Susceptible.

In nature rabies is confined to a comparatively small number of species of animals so far as it has come under observation. It is essentially a disease of canine animals and is transmitted by them to a number of other animals. The artificial transmission of rabies, however, is possible among a wide range of mammals and it may be assumed that no mammals are insusceptible. Birds are also susceptible. Most workers emphasize the point that bird rabies has an unusually long duration. Some go the length of describing spontaneous cures. Instead of the usual duration of 4 or 5 days, well-developed rabies in birds may last for two or more weeks.

Cold-blooded animals are more difficult to infect, but success has been attained with frogs, and the true nature of the affection apparently shown by transmissibility from frog to mammal.

Among the mammals some differences of susceptibility are met with. The muridæ, for example, are comparatively easily infected by subcutaneous inoculation of fixed virus (see p. 203) and with some viruses, such as the Sassari, this susceptibility is extremely pronounced. Man, on the other hand, is very little susceptible to subcutaneous inoculation of fixed virus. Some mammals, although susceptible, are said to show degeneration of the virus with passage even with subdural inoculation. Thus street virus (see p. 203) with passage in monkeys was at one time thought rapidly to lose instead of gain virulence. It was shown, however, by Acton and Harvey (1913) that this was not the case and that street virus would become a fixed virus in the monkey, as in the rabbit.

Pathological Anatomy and Histology.

In an animal which has died of rabies, there is marked emaciation of the body. A very characteristic and highly suggestive appearance in rabid dogs is the occurrence of all sorts of strange material in the stomach, such as straw, wool, chewed wood, hair and leather. The mucous membranes of the whole gastro-intestinal tract and of the related glands are congested and hæmorrhagic. This is not due, according to Puntoni (1928), to injury inflicted by foreign bodies, but is associated with the elimination of the virus by these surfaces. None of the naked-eye appearances can be said to be pathognomonic nor are they even invariably present.

Much stress was laid at a time previous to Negri's discovery of the specific bodies which bear his name, on the changes in the central nervous system associated with rabies, and especially the cell aggregations around small blood-vessels and ganglion cells. These changes are mainly of an inflammatory character, but are more constant and more marked in some other diseases, as for example, in epidemic poliomyelitis, owing in all probability to insufficiency of time for their development in rabies. These appearances are :

(1) *Vascular.* The small vessels of the central nervous system show narrowing of lumen and enlargement of the endothelial cells, with thickening of the walls. There is a marked round-cell infiltration of the adventitial lymph spaces, which sharply defines the vessels.

(2) *Neural.* Marked changes are found in cases of rabies in and around the ganglion cells of the cerebrospinal and sympathetic ganglia. The ganglion cells are encircled or completely replaced by a collection of round cells, which are regarded by some as due to the proliferation of the ' satellite ' cells (neuroglia), and by others as a small round-cell infiltration. Probably both enter into the reaction. The ganglion cell shows swelling, with eccentricity of nucleus, and undergoes degeneration, with chromatolysis, karyolysis, ultimate disintegration and neuronophagia. These perivascular and periganglionic cell infiltrations give rise to the so-called ' rabies nodes '. They are often very restricted in their distribution and may occur side by side with perfectly normal tissue.

(3) *Salivary glands.* Beyond the œdema, hyperæmia and the small hæmorrhages which are found in other tissues, these do not show any special features.

All of these appearances have greatly diminished in importance with the prominence assumed by the ' Negri body '. It was discovered in 1903 (Negri). Affections other than rabies have been stated to give rise to the Negri body, but there is now little doubt regarding its specificity. Discussion, however, still centres around questions of whether it is the parasite itself, a degeneration developed around the parasite, or reaction on the part of the nucleus of the nerve-cell to the parasite. The Negri body is found most constantly and in its most highly developed form in the ganglion cells of the hippocampus major, although it is also found to a more limited extent in the pyramidal cells of the cortex, the Purkinje cells of the cerebellum, and in the ganglion cells of the pons, medulla, spinal cord and sensory ganglia. It is the hippocampus major which is now regularly examined for the histological diagnosis of rabies, because it is here that Negri bodies are most readily found. They are situated in the nerve-cell cytoplasm or in the protoplasmic processes, especially in the central pyramidal layer, and there may be one or more in the same cell. They are round or oval, and vary in size from 1 to 15μ or more. The smaller and the less spherical, more elongated types are found in the cell processes, but size depends also to some extent on the size of the animal affected. They are particularly large in the horse and ox, and in the later stages of the infection. The nerve-cell itself shows little alteration of either nucleus or cytoplasm as a consequence of the presence of the Negri body. Staining of the eosinophilic bodies is easily accomplished by ordinary methods, although Mann's methyl blue eosin method is superior to all others. The bodies can also be seen in an unstained condition by examination of teased hippocampus in dilute acetic acid. With Mann's stain the Negri body shows internal structures which stain less intensely than the generally red-coloured matrix. These internal structures are fewer in number the smaller the Negri body, and may, in the larger forms, be as many as 20 to 25, of varying size. Each of these ' inner bodies ', as they are called, contains an intensely staining corpuscle. Questions naturally arise as to the nature of the Negri body and of its inner bodies. These are considered later. With continued subpassage of the street virus, and consequent approach to fixed virus character, the Negri bodies become smaller and smaller and ultimately cannot be found.

Staining of the Negri body. A large number of methods have been employed, of which the slower are to be recommended in preference to the more rapid. Most of these depend upon the acidophilic character of the body. Mann's method, when successful, gives beautiful results and has been very generally used. Heidenhain's iron hæmatoxylin method has proved very useful for bringing out details of inner structure. For details of application see Vol. IX.

Nature and Properties of the Virus.

The properties and the mode of action of rabies virus were very thoroughly worked out originally by Pasteur himself, and knowledge on this subject, except for the discovery of the Negri body, and the alignment of the virus with other neurotropic filterable viruses, has not been greatly added to since his time.

Street virus and fixed virus. The terms ' street virus ' and ' fixed virus ' were applied by Pasteur and had very definite connotation. The street virus was simply the infecting agent of rabies, which is met with in the infecting fluids or tissues of animals developing rabies under natural conditions, and was proved by its transmissibility to be a living entity. Thus the saliva of the rabid dog contained street virus, and so did the tissues of the central nervous system of the animal. It was found that a suspension of the brain of an animal dead of street rabies when injected subdurally in the rabbit gave rise to symptoms in 15 to 20 days. With continuance of subdural inoculations from rabbit to rabbit, and more rapidly if the animals used were young, the incubation period of the disease was progressively shortened until after some 50 passages, a point was reached at which the symptoms appeared in seven days. This incubation period might be shortened by selection to a 6-day period and by other means, but it remained constant at this 6- to 7-day figure. The virus, obtained by passage through a succession of rabbits inoculated subdurally or intracerebrally, was called fixed virus by Pasteur.

It had certain properties, other than incubation time, which differentiated it from the street virus of its origin. Shortly stated, the differences of importance are reduced pathogenicity for man by subcutaneous injection, non-occurrence of Negri bodies, and shortening of the incubation period during the process of fixation. The fixed virus character appears to be permanent and reversion to street virus has not been effected. If we consider the repeated subpassage on rabbit-brain to which the street virus is subjected in the course of production of fixed virus, these differences are similar to what appear with the habituation of any microbe to a new medium of growth. The view generally held is that this neurotropic virus, in becoming fixed, has become more habituated to growth in association with a cellular nervous tissue, and less able than street virus to establish infection by subcutaneous implantation. Remlinger (1928), impressed by the fact that rabies saliva when inoculated under natural conditions is frequently found to be infective even in very minute doses, inclines to the view that the germ exists in the saliva in a different form to that in which it is present in the brain. The difference between street and fixed virus appears to be one of degree. There is little difference in the duration of the disease once developed, whether produced by street virus or fixed virus, and the concentration of the virus in the brains of street virus and of fixed virus animals appears to be identical, as judged by the dosage required to produce the disease subdurally (Harvey and Acton, 1923).

Neurotropic character of the rabies virus. The neurotropic character of the rabies virus was very early established. That the central nervous system contains the infective agent may be proved by injection into animals. The delay, which may be considerable, in development of symptoms and the variation in incubation period with distance of bite from the brain, speak strongly in favour of development in and propagation along nerve paths. Blood, in sharp contrast to nerve substance, remains incapable of transmitting the disease to animals. The crucial experiments which proved the mode of propagation of the virus and its restriction to the nerve paths were those of di Vestea and Zagari (1889), in which they showed that, with injection of fixed virus suspension into the sciatic nerve, the cauda equina became virulent before the medulla and that with subdural inoculation over the cerebrum the medulla became virulent before the lumbar cord. The salivary glands, the pancreas, intestinal glands and some others with their secretions become infective after the central nervous system, presumably from the passage of virus down the nerves to nerve-cells in these glands. The saliva, however, is the only secretion in which the virus of rabies is found with any degree of certainty or constancy. Manouélian and Viala (1924) describe numerous nerve-cells situated in the lingual and buccal mucosa which are placed very superficially and are irregularly distributed. With the exposure of these by trauma, one can understand that the virus might escape from affected nerve-cells and render the saliva infective. The infectivity of the saliva, moreover, might, on this view, be only occasional. The experiments of Cruickshank and Wright (1914) seem to demonstrate this occasional want of infectivity of the saliva.

The Negri body. The specific inclusions in the ganglion cells of the central nervous system, the Negri bodies, which are present in this disease, are characteristic of street virus and are not present, or very sparsely present, in rabies brought about by fixed virus. This difference, however, is not very material as it depends in all probability on the need of a certain amount of time for the development of the Negri body, such as is given by the long incubation of street rabies, and not necessarily to any essential difference between fixed virus and street virus.

The opinion of Negri himself on the nature of the specific ganglion cell inclusions discovered by him was that they were protozoa, and were the actual cause of the disease. The belief in the protozoal nature of the Negri body is supported by its occurrence within the protoplasm of the nerve-cell, its attainment of a considerable size, and its possession of a definite internal architecture, together with the absence of signs of degeneration of cytoplasm or nucleus of the host-cell. The whole body may, according to this view, be the parasite, or the internal structures may be the parasites embedded in a matrix.

Another view altogether is that the virus of rabies becomes surrounded by altered cell material to form the Negri body and it has been classed, from this point of view, with the *Chlamydozoa*, as defined by Prowazek. Acton and Harvey (1911) considered that the Negri body was simply a derivative of the nucleolus. This view they based on the identity of the staining reactions of the Negri body and the nucleolus. The appearance of the Negri body does not conform to that of any known protozoal parasite. It is not found in the infective saliva of the rabid animal, nor in some of the undoubtedly infective portions of the nervous system. The filterability of the rabies virus is a difficulty which has to be got over if the theory of protozoal nature is to be accepted, and is avoided by assuming that all stages of development exist between wholly invisible forms of the protozoon, through the minute forms which can be seen in protoplasmic nerve processes to the fully developed large Negri body in the cytoplasm of a nerve-cell of the hippocampus. The size of the infective agent was gauged by Remlinger at a very low figure, owing to the fact that it required centrifuging of a suspension of rabies brain for an hour at 1,000 revolutions before the supernatant fluid could be obtained free from virus. The parasite was given the name *Neuroryctes hydrophobiæ* by Calkins.

Still another view has been put forward as to the specific microbial identity of the Negri body. Levaditi and his co-workers regard it as an organism analogous to the so-called *Encephalitozoon cuniculi,* belonging to the microsporidia. The Negri bodies would, according to this view, represent the visible pansporoblasts and cysts of the specific protozoon, which they have called *Glugea lyssæ.*

Encephalitozoon rabiei. This parasite was described by Manouélian and Viala (1924) as occurring in the brain, spinal cord, ganglia and salivary glands, within the nerve-cells. They describe the active phase as consisting of elongated fusiform or pyriform bodies, averaging 1 to 2μ in length, but frequently smaller and just at the limit of visibility. They consider that Negri body formation is due to the degeneration of these intracellular forms and is brought about by their agglutination into masses which ultimately coalesce.

Passage virus bodies. The Negri body disappears from the cells of the hippocampus along with the transformation of street virus to fixed virus. Lentz (1909) has, however, described bodies which he believes to be specific in rabies passage rabbits. They lie between the ganglion cells, and are of somewhat oval form, take the eosin (oxyphile stain), and contain ill-defined structures which stain dark blue. Their specificity has been called in question and they have been regarded as leucocytes and as degeneration products.

Coccus-like bodies. The presence of coccus-like granules, other than minute Negri bodies, has been described as present in rabies brains and not in normal brains. This observation indeed goes back to the time of Pasteur who, however, laid no particular stress on the observation. It has received a certain amount of support, but the description of a body as ' coccus-like ' is very suggestive of chemical precipitation.

None of the generic and specific names applied to the rabies organism or to the Negri body—*Neuroryctes hydrophobiæ* (Calkins), *Glugea lyssæ* (Levaditi), *Encephalitozoon rabiei* (Manouélian and Viala)—can be considered to have established itself as generally acceptable. At the International Rabies Conference held under the auspices of the League of Nations (1927), it was placed on record that the Commission, from the evidence now available, were not in a position to decide whether the virus was a protozoon or a bacterium, nor whether ' Negri bodies represent a stage in the evolution of a living microsporidium or are produced by cellular degeneration '.

Evidence for an Ultramicroscopic Organism and of Filterability.

The characters which in the aggregate combine to place the causative agent of rabies among the ultramicroscopic organisms are those which are to a greater or less extent common to the group of filterable viruses as a whole, such as failure to demonstrate the organism in known infective material, failure to cultivate the organism in the absence of living cells (see, however, p. 209), capability of preservation in pure glycerin, the occurrence of cell inclusions in tissues which are specific for the disease, as exemplified by Guarnieri bodies in variola, trachoma bodies, and the Negri bodies of rabies, and finally the filterability of specifically infective material through Berkefeld or Chamberland candles. This last is perhaps, when taken in conjunction with invisibility, one of the most important descriptive characters for the ultramicroscopic virus class. It is not absolute, as for example in the case of some spirochætes which, with visibility, still show filterability.

It is not very remarkable that the filtrate of rabies material should still be infective, even if only a very minimal amount of virus succeeds in passing the filter, for the unfiltered material can be diluted to an extraordinary extent and still be capable of giving rise to the disease in subdural inoculation. Thus, Harvey and Acton (1923) found that infection was still taking place with subdural inoculation of 0·2 c.cm. fixed virus suspension in dilution of 1 : 40,000.

The tests of filterability of any virus must be very rigorous to be convincing. Their success depends on the medium in which the virus is contained and this may be very difficult with a virus contained in blood plasma, or, as is probably the case for rabies, a virus which is intracellular. The filter used should be new, a Berkefeld candle of type V, N or W, or a Chamberland F or B ; the filtration should be conducted under as low a pressure or suction as possible and as quickly as possible ; the filtrate should be tested in suitably large quantity by subdural inoculation for the demonstration of the presence of virus. Most important of all is the preliminary testing of the filter used with known non-filterable organisms. With such tests it has been found that rabies virus can pass through candles with pores of diameter 0·2 to 0·8μ, and the method is used for testing suspected rabies brain which has undergone putrefaction and cannot be used directly for diagnostic inoculation. There are varying ideas of the degree of permeability of the filter which allows of passage of the rabies virus. Remlinger restricts this to the V type of Berkefeld filter, but much depends here on the filtration pressure applied. It is essential that special attention be paid to the fine trituration of the rabies material for filtration. Clogging, however, by fatty globules is very apt to take place.

Action of Physical and Chemical Agents on the Virus.

The rabies organism, whatever it may be, is in one way or another bound up with nerve material and may very probably be intracellular. This fact has to be taken into account in all pronouncements regarding the effect of physical or chemical action on the virus.

Physical agents. Of physical agencies temperature follows much the same course as in the case of any micro-organism—that is to say, temperatures of 45° C. and over, kill, and in shorter times the higher the temperature. It takes about 24 hours at 45° C. to sterilize rabies brain. Low temperatures have comparatively little effect on viability or virulence. The most important of the physical agents to consider as regards its effect on rabies virus is that of desiccation. Desiccation has been used to attenuate rabies virus from very early times. It was the original method used by Pasteur himself for the preparation of fixed virus rabbit-cords for the vaccine of treatment. Harvey and McKendrick (1907) contended that drying does not so much attenuate as diminish the total quantity per unit of volume by killing the virus from the surface inwards. In the absence of oxygen, however, desiccation would appear to have much less action on the reduction of virulence. Powdered dried vaccines are said to maintain their virulence up to 3½ months when all air contact is avoided, as for example, by keeping them in cocoa-butter. Desiccation is used not only as a rabicidal agency but also as a preservative. The determining factor is temperature : if rabies material be quickly dried at the low temperature given by carbon dioxide snow it can be preserved in the completely dry state for months and possibly indefinitely. This is the basis of Harris's method of preparation of rabies brain for use as vaccine. Other physical agencies such as, light, ultraviolet- and X-rays, may reduce virulence, and do so, in all probability, to the extent to which they can penetrate brain material and kill the virus.

Chemical agents. Various chemical substances have been used to preserve the rabies virus or to kill the virus in the preparation of vaccine. Glycerin holds a special place as a preservative of neurotropic filterable viruses. Rabies material can be preserved in glycerin for a considerable period of time if the temperature is low. This fact is made use of for the despatch of test material to a distance. The glycerin also prevents putrefaction, and so the material may be used for subdural inoculation in animals without liability to the production of infection by extraneous organisms. Glycerin has also been used in the method of Calmette for the preservation of rabies vaccine material so that it can be used at a much later date than its actual preparation.

Phenol is an antiseptic which has been tested on practically every living pathogenic organism. The rates at which it kills the rabies virus are very variously given by different workers, and these differences are doubtless due to the conditions as regards trituration and temperature to which the test material has been subjected in the experiments. Phenol has a special importance as an antiseptic : it was first used by Fermi for vaccine purposes in 1906 to reduce the virulence of the rabies virus and later by Semple (1912) to kill the virus. It is found that 1 c.cm. of a 2 per cent. fixed virus suspension, subjected to the action of 1 per cent. phenol for 24 hours at 37° C., does not transmit rabies when injected intramuscularly. This carbolized vaccine is now used throughout India, and is also used elsewhere. Another vaccine-sterilizing method is that which uses ether as the antiseptic. Its action may vary somewhat with different viruses. According to Cunningham, Nicholas and Lahiri (1926), the Kasauli virus is in almost every instance sterilized by ether in 84 hours. Other strains appear to be more resistant. The Paris strain, for example, is much more so than the Kasauli strain (Cunningham, Nicholas and Lahiri, 1928). Other antiseptics than those mentioned have been tested for sterilizing action on rabies virus.

Putrefaction has a comparatively slow action on the viability of the rabies organism contained in the brain of a rabid animal. The fact is important because in cases of doubt whether a biting animal has been rabid or not, that doubt may still be set at rest even after an animal has been dead for some time.

The effect of various substances such as, quinine, arseno-phenylglycine, iodine, dichlorbenzidine, toluidine and methylene blue have been tried. But the results so far have been poor.

Artificial Culture of the Virus.

The rabies virus cannot grow in the media ordinarily used for bacterial culture, but various special media into the composition of which aqueous humour, cerebrospinal fluid, serum, ascitic fluid, living tissues, &c., entered, have been claimed to give a growth. Special methods have also been applied, such as that of cultivation in collodion sacs in the abdomen, cultivation in the brain of a freshly killed animal, cultivation by placing a normal brain in juxtaposition with a rabies brain, and so on. Success has been claimed for such procedures also, but the authors have not satisfactorily excluded the possibility of simple diffusion of virus nor the possibility of simple dilution without growth of the original material. Dilution of material can, as we know, be carried to very great lengths, and yet the suspension so obtained still be capable of giving infection by subdural inoculation.

The experiments on culture which have attracted most attention are those of Noguchi (1913) in which he applied the method which he had proved successful for the cultivation of spirochætes. The culture is in plasma or ascitic fluid containing a piece of sterile fresh rabbit-kidney under a layer of paraffin. After incubation at 37° C. for 7 to 10 days it was found that, although no change had taken place in the fluid macroscopically, there had developed microscopically fine chromatin-staining granules and coarser pleomorphic bodies. Subculture of these apparent growths could be made, and in one case, where the subculturing procedure had occupied seven months, it was successfully carried to the twenty-first generation. Noguchi also described uninucleated bodies in these cultures which were furnished with a membrane. But these findings, like those which preceded them and those which have come after, have not survived the test of time or the objection that they were due to the production of purely physico-chemical changes in the medium used, of diffusion of virus, retention of infectivity over a long time and in great dilution, and above all, the objection that similar appearances can in some cases be produced in cultures by the use of normal instead of rabies brain. In fact there is no conclusive evidence that the rabies organism has as yet been cultivated on artificial media even in the presence of living cells.

Transmission Experiments.

In nature, one mode and one mode only stands out pre-eminently as the cause of rabies and of its continuance, and that is biting, by which infective saliva is introduced subcutaneously into the bitten animal.

The saliva of an infected animal may contain the virus for a few days before the appearance of symptoms. Various modes of prevention of infection after biting have been the subject of much experimentation, and there seems little doubt that the virus must remain localized for a short time at least. Dogs inoculated at the tip of the tail with rabies virus could still be saved from rabies by amputation at the root within 12 hours, and enucleation of the eyeball, which had been similarly inoculated, served to prevent onset of rabies if carried out within 24 hours. Cauterization experiments after infection have been much less successful.

Subdural or intracerebral injection. With a careful procedure, in which, by slow withdrawal of the injecting needle and the maintenance of positive pressure, reflux is prevented, it may be said that an injection of 0·2 c.cm. of a thick suspension of fixed virus material will in all ordinary laboratory animals result in 100 per cent. infection with rabies. The degree of failure to attain this percentage represents the degree of ineffectiveness of the technique used. Of course, the strength of the suspension is a factor in infection. Harvey and Acton (1923) found that escapes began to take place with dilutions of 1 : 1,000, but that infection could still take place with dilutions as high as 1 : 40,000.

Intra-ocular injection. This mode of injection is, because of its more difficult technique, a little more uncertain than intracerebral.

Intramuscular and intraneural injection. If such injection be practised in the neck muscles the proportion of infections obtained is high, although not so high as by the subdural method. This method is of great use for experimental work on rabies immunization, as it has not the extreme severity of the subdural test. It is also useful where the material available for testing is contaminated with bacteria, as is often the case when it is sent to the laboratory from a distance and during hot weather. The intraneural injections are uncertain.

Intravenous injection. An intravenous injection is very uncertain in its production of rabies.

Intraperitoneal injection. This is so uncertain that it is never employed.

Subcutaneous injection. Subcutaneous injection is the laboratory procedure most closely allied to the mode of transmission of rabies by biting in nature. It is less certain than intramuscular injection and may be expected to give rise to infection in about 50 per cent. of cases. In nature, where the injection is subcutaneous, the passage of the tooth of the animal through clothing by which it is cleaned of saliva, greatly diminishes the possibility of infection. The subcutaneous injection of the muridæ, and especially with particular viruses (e.g. Sassari), is much more successful than in rabbits. The fact that fixed virus may be injected subcutaneously into man without appreciable risk is the basis of preventive methods of treatment which depend on the use of living virus.

Scarification. Many consultations on the necessity for treatment of human beings have reference to the occurrence of a scratch on the bare skin rather than a bite by the tooth of a rabid animal. Babes and Vasilu

(1911) could not produce rabies in animals by rubbing street virus on superficial scarifications ; they concluded from their extended experiments that no treatment was needed in the case of licking by rabid animals of wounds which were 48 hours' old. There is no doubt that infection by scarification of skin is rarely successful under experimental conditions (Remlinger, 1928). Scarification of the cornea and deposition of fixed virus is on the contrary almost uniformly successful (Kraus and Fukuhara, 1909).

Deposition of virus on skin and mucous membrane. The case of skin or mucous membrane with even trivial open lesions is the same as that of scarification. Can rabies virus infect through intact skin or mucous membrane ? Our own opinion is that under natural conditions it cannot, although there are many contrary opinions.

Ingestion. Experimentally rabies has not been transmitted to animals by ingestion of infective rabies material nor by feeding with the flesh or milk of the rabid animal. Such a mode of infection may be excluded.

The facts of failure and success by various modes of introduction of virus are consistent with the view that contact of the virus with susceptible nervous tissue is a necessary occurrence for infection.

Specific Prophylaxis.

At the outset it may be pointed out that antirabic immunization is in practice not pre-infectional but post-infectional. It is, therefore, a curative procedure in the sense that it extinguishes infection before the latter has given rise to symptoms. It is not analogous, therefore, to the ordinary vaccination against smallpox or to the prophylactic use of vaccines for the enteric group of diseases, but rather to the procedure that employs vaccination as a means of aborting infection which may already have been contracted. Precisely the same reasoning applies in this case to the use of vaccinia virus as to rabies virus. The incubation period of smallpox is sufficiently prolonged to admit of the acquisition of a vaccinia virus immunity to that disease within the time available to onset of symptoms. In rabies the time available is considerable, and may vary from 17 days for individuals bitten on the head to periods of 2 or 3 months for bites on the lower extremities. During this incubation period of street virus, active immunization with fixed virus is carried out with a view to aborting the infection. Various methods have been employed to this end both as regards the nature of virus employed and the duration of its employment. The vaccine may consist of living or dead virus ; cord or brain material ; fixed virus of the rabbit or of the monkey ; vaccine alone or combined with antiserum ; it may be glycerinated, carbolized or etherized. It may be administered in an Institute or, in certain cases may be sent out by post for use. A consideration of all these modes is not possible here, and it suffices to say that they are all in greater or less degree variations of the original Pasteurian method, and they may, in spite of their great variety, all be denominated Pasteurian. Pasteurian vaccines are divided into two main classes, according as they contain living or dead virus. The success of dead* virus vaccines, both in animal experimentation and in extended application to human beings, disposes of the contention that immunity to diseases produced by filterable viruses can only be brought about by the use of living antigen.

The mode of preparation of certain selected types of antigen may be given without entering into the details of administration.

1. *Desiccated antigen* (Pasteur). (1) Remove the medulla of a fixed virus rabbit which has been killed not earlier than the ninth day after subdural inoculation. (2) Make a suspension of the medulla by triturating in 1 c.cm. 0·85 per cent. salt solution. (3) Trephine a rabbit. (4) Inject 0·2 c.cm. suspension subdurally. (5) Return the rabbit to its cage. (6) Take an inoculated animal daily, which has shown paralysis and been killed not earlier than the ninth day after inoculation. (7) Expose the spinal cord and medulla with aseptic precautions. (8) Sever the cord just below the medulla. (9) Snip off a portion into nutrient bouillon. (10) Incubate 24 hours as a test of sterility. (11) Remove the spinal cord intact. (12) Divide it into two equal pieces and suspend each separately by sterile silk threads in a sterile glass jar containing sticks of pot. hydroxide. (13) Place the fourth ventricle and medulla in a sterile dish and use for continuing the passage inoculations in the rabbit. (14) Make a post-mortem examination of the rabbit for intercurrent disease, which, if present, will necessitate rejection of the material. (15) Keep the jars in which the spinal cords are to undergo desiccation at 22° C. (16) Make a suspension of 1 cm. of cord at the various periods of drying in 3 c.cm. of 0·85 per cent. salt solution. Such doses are administered daily over varying periods. A modification of this method is that of Calmette, in which cords, dried for varying periods, are subjected to the action of glycerin.

2. *Fresh antigen* (Hoegyes). (1) Remove the medulla of a fixed virus rabbit. (2) Weigh. (3) Grind up thoroughly in a sterile mortar with 0·85 per cent. salt solution to make a 1 in 100 suspension. (4) Make up a series of dilutions of the suspension with 0·85 per cent. salt solution, 1 in 10,000; 8,000; 6,000; 5,000; 2,000; 1,000; 500; 250.

3. *Carbolized antigen* (Semple). (1) Remove the whole brain and medulla of a fixed virus rabbit. (2) Weigh. (3) Grind up thoroughly in a sterile mortar with 0·85 per cent. salt solution containing 1 per cent. carbolic acid so as to make a 2 per cent. suspension. (4) Filter through sterile gauze, placed in a fine-mesh strainer. (5) Place in incubator 24 hours to allow the carbolic acid effectually to kill the rabies virus. (6) Dilute with an equal volume of 0·85 per cent. salt solution. (7) Place in the ice-chest till required for use. (8) Dilute again at time of use with an equal volume of 0·85 per cent. salt solution. (9) Administer in doses of 4 c.cm. daily for 14 successive days.

4. *Etherized antigen* (Alivisatos). The whole brain is immersed in ether for 72 hours at the temperature of the ice-chest. Gray matter is as far as possible separated and is alone employed. An emulsion is made in 0·8 per cent. salt solution. The modification of this vaccine as now used by Hempt is also carbolized and is a dead vaccine.

Neuroparalytic accidents. Brief mention must be made of these, which are a source of anxiety to all who have to do with antirabic treatment. It was first shown by Remlinger (1905) that certain paralyses, chiefly of the lower extremities, developed in a certain proportion of cases under treatment for rabies. The paralyses were practically always recovered from, and had but little resemblance to the syndrome of rabies itself. The explanations offered from various sources of these occurrences were that they represented an abortive rabies which had been partly extinguished by the antirabic treatment already carried through ; that they were manifestations of the action of a rabies toxin and not of the living virus as such ; that they were due to a non-specific protein intoxication, or an allergic action of the foreign nerve substance injected along with the rabies virus. Harvey and Acton (1923) showed that paralysis in rabbits could be produced by treatment with normal brain suspension. Schweinburg (1924) and Stuart and Krikorian (1928) have obtained similar results. The latter have also shown that this toxic action is destroyed by phenol. With the introduction of the use of fresh virus or of intensive treatment with cords which had been scarcely desiccated at all, these manifestations took on in some cases a fatal and somewhat altered character. Some of the symptoms too were referable to a more central origin than before. In fact, the disquieting suspicion arose that these new cases differed from the old ' paralytic accident ' in being cases of ' râge laboratoire '. The mortality among patients undergoing antirabic treatment was not affected by the deaths which took place, owing to their extreme infrequency. With the adoption of a killed carbolized vaccine, these accidents have almost, if not entirely, disappeared.

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* The test of death adopted in practice at Kasauli, India, extending over some 500 samples of Semples' vaccine, was the intramuscular injection of 1 c.cm. in rabbits. In no case did a rabies infection occur.

Results of treatment. Comparatively exact knowledge is now available of the percentage mortality amongst the treated. McKendrick (1928) summarizes evidence obtained from cases treated at Kasauli (India) from 1900 to 1925. The statistics show that as good results were obtained by the use of carbolized vaccines (mortality 1·16 per cent. in 84,844 cases) as with either dried cords (mortality 1·81 per cent. in 5,141 cases) or dilutions of fresh nerve substance (mortality 1·61 per cent. in 8,435 cases). From an analysis of figures relating to 20,000 Indian patients, the following conclusions are arrived at :

(1) The chance of infection increases with the number of tooth marks.

(2) A deep bite is about five times as dangerous as a superficial bite.

(3) Interposition of clothing reduces the mortality in the ratio of 6·6 to 1 : cauterization in the ratio of 4 to 3.

(4) The length of the incubation period is not affected either by number of bites, depth of bite, interposition of clothing or cauterization, and is thus independent of the effective dose.

(5) The benefit of treatment as indicated by the advantage of early arrival is demonstrated in cases whose periods of incubation are relatively short (head and arm bites) both by a reduction in mortality and by a lengthening of the incubation period.

(6) The period of incubation is primarily dependent on the site of inoculation (head, 27 days ; arm, 32 days ; leg, 64 days) ; it is independent of the magnitude of the effective dose ; it is prolonged as a result of Pasteurian treatment.

There is considerable divergence of opinion on what is the mortality amongst the untreated, by which the success of treatment may be gauged. It is not possible to enter here on the various estimates of untreated mortality, but it seems to us probable that the estimate of Babes of 5 per cent., or even less, for a general population of persons at risk is a reasonable statement. This estimate is necessarily arbitrary owing to the variation in constitution of the population upon which estimates are based in respect of the extent to which they are ' at risk '. Where special classes of cases such as head cases, wolf and jackal bites, multiple bites, deep bites on bare skin and so on, are in excess, the estimate would be higher. As regards the mortality amongst the treated, it is well known that considerable numbers of those attending for treatment at Pasteur Institutes, especially in these days of well-informed opinion and facility for treatment, are scarcely, or not at all, at risk. There is really no figure for the untreated which would be comparable with that of a Pasteur Institute, as every Institute has a different population constitution. The estimate of mortality among such populations may easily vary from as low as 1 or 2 per cent. up to 10 per cent.

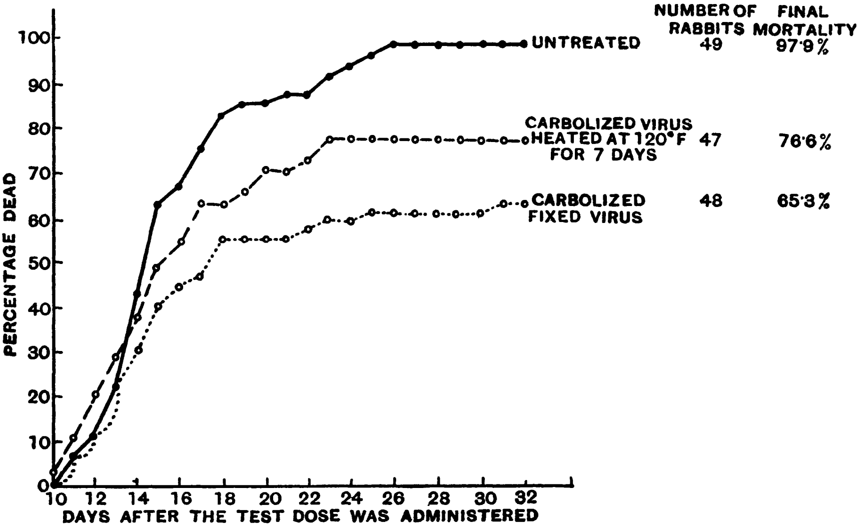
It may be well to conclude with insistence upon the necessity of those antirabic measures which concentrate upon the outbreak of rabies among dogs, as being the most rational and the most effective of preventive measures, and to consider the ordinary method of treatment only as a second line of defence.

Immunity.

Natural immunity. Certain animals such as reptiles and fish are insusceptible to rabies. Among mammals none show insusceptibility to infection. Birds, especially old birds, are difficult to infect. Some of the claims to the existence of a natural immunity are of doubtful character and statements regarding both natural resistance and acquired resistance in animals such as rabbits, and especially dogs, have to be scrutinized in the light of difficulties in the technique of transmission (Harvey and Acton, 1923). Also it has to be remembered that many localities are unsuitable for the transmission of infection owing to lack of nerve elements.

Acquired immunity. The principles of acquisition of immunity to rabies infection are precisely those which have been applied in bacterial diseases. It was as a result of his researches into the protection which could be afforded to animals against anthrax and fowl cholera that Pasteur was able to establish the possibility of antirabic immunization. As in those diseases, he elaborated a method of producing a virus of constant strength and of controlling its attenuation. This standard virus is the fixed virus to which we have already referred. The attenuation of the virus so as to provide a gradation of virulence from zero up to full degree was effected by drying. This process was, probably, a method of reducing the quantity of living organisms present in the antigenic material. The method adopted by Pasteur for active immunization, which in its various modifications is that commonly employed at the present day, was not the one of widely spaced doses, such as is commonly used for bacterial immunization, but of daily dosage. The dosage might even be repeated more than once on the same day.

The main proof of the efficacy of active immunization in rabies is given by the significantly larger proportional survival of animals treated with rabies antigen than of animals not so treated, or of animals treated with non-specific antigen. Controlled experiments, on the scale necessary to bring out differences, are furnished by Harvey and Acton (1923) on 68 monkeys. These gave 3 survivals out of 19 treated with Hoegyes' intensive dilution method, 3 survivals out of 15 treated with 4 per cent. fixed virus, no survivals out of 15 treated with 4 per cent. normal brain suspension and one survival out of 19 untreated control monkeys. McKendrick's experiments (Harvey and McKendrick, 1923) on the same subject are shown in the following chart.



Very numerous experiments, however, commencing with those of Pasteur himself, had already established the simple fact of immunity.

The serum of the immunized animal should, on the analogy of bacterial immunization, give evidence of antibody formation. Controlled experiments (Kraus, Keller and Clairmont, 1902) have shown that the serum of the normal rabbit is not rabicidal, whereas that of the immunized rabbit is. The proof of rabicidal action is given by testing the virulence upon rabbits of the mixtures of sera and virus which have remained in contact at suitable temperatures. There is no sufficient evidence for a cross immunity given by rabies virus to any of the other allied filterable viruses. The complement-fixing properties of an antirabic serum are also difficult to demonstrate on account of the large amount of cerebral matter present, and are largely non-specific. A final argument in favour of active immunization in rabies is given by the results obtained in the case of the enormous numbers of individuals who have been treated for rabid dog bite and who have escaped infection.

Passive immunization in rabies is not, any more than in most bacterial diseases, a procedure of any value. That antirabic serum has any antitoxic character is very doubtful in view of the difficulty of proving even the existence of a rabies toxin, and no facts are available to show that rabies, once it has developed in man or animal, can be controlled by the use of serum or any other means. Such a serum, however, has been used in conjunction with antigen as a serovaccination method of treatment.

The question of the existence of a rabies toxin is important in connection with active and passive immunization. The existence of a rabies toxin has been assumed as an explanation of the phenomenon of the 'paralytic accident'. One theory of active immunization was based on the supposition that the toxin introduced with the material inoculated is rapidly absorbed and produces its effect upon the central nervous system, whereby the latter is rendered unassailable by the rabies organism, whether that of the original infection or of the subsequently injected material. Once this is accomplished, antibody production or resistance to the organism results in the ordinary way. If appreciable amounts of an extracellular toxin were produced by the rabies organism, it might be expected that injection of large amounts of rabies virus material would produce symptoms of specific intoxication. Harvey and McKendrick (1907) showed that the injection of a 10 per cent. suspension of fresh fixed virus subdurally in an animal in amounts of 1 c.cm. will not produce symptoms of intoxication, nor does the disease develop any sooner than after the inoculation of 0·2 c.cm. of a 0·2 per cent. suspension. So also the injection of relatively enormous doses of virus subcutaneously or intraperitoneally failed to produce any symptoms of intoxication. The existence of an endotoxin of some sort may be argued from the general nature of the symptoms of rabies, but the absence of signs of intoxication following the injection of large quantities of rabies nerve material shows that, if present at all, it must be in very small quantity.

There is finally the theory, which has received recent support, of a purely localized tissue immunization to rabies infection. The modern theories of neurotropic, dermotropic, enterotropic and other histotropic infections and immunization has brought the possibility of an acquired cerebral cell immunity or resistance into greater prominence. Kraus and Maresch found that fowls, subjected to prolonged treatment with rabies virus, showed no protective substance in their serum, and yet were immune to infection, in marked contrast to control untreated birds. Many other experiments have in recent years been carried out to demonstrate local tissue immunization, but no definite proof is as yet forthcoming. It is at present impossible to decide whether the immunity conferred by active immunization is antimicrobic, antitoxic, anti-endotoxic or histiocytic in character.

Virus of Kobayashi.

By S. P. Bedson.

This virus was obtained by Kobayashi (1925) in the epidemic of encephalitis in Japan in 1924, in which 4,000 people died with a mortality of over 50 per cent.

Kobayashi inoculated over 200 rabbits with different material—brain, other organs, cerebrospinal fluid, blood and nasopharyngeal washings. The great majority of these animals remained perfectly well, but occasional one died without having shown any symptoms, and from one of these inoculated with a brain emulsion this virus was obtained by passage. The rabbits of the first and second passage died without symptoms, but the third and fourth passage animals developed a quite definite encephalitis, which proved fatal, and subsequent transfers were almost invariably successful. This virus was shown by Kobayashi to be virulent for the dog, cat, guinea-pig, rat, mouse and pigeon, as well as the rabbit. It was moderately resistant to glycerol, for after two months in 50 per cent. glycerol active virus was still present, though considerably reduced in quantity. It did not pass through Berkefeld N or Chamberland L2 or L3 filter candles. Kobayashi recognized that this virus resembled rabies virus, from which, however, it apparently differed by only infecting the dog when introduced subdurally, in localizing chiefly in the brain in infected animals, and in the prolonged course run by experimental disease. Cowdry (1927) has made a comparative study of the virus of Kobayashi and that of rabies and from the similarity of the lesions produced in the rabbit's brain by these two viruses and from the result of cross-protection experiments in rabbits, he has concluded that the two viruses are identical.

Virus of Koritschoner.

By S. P. Bedson.

This virus was isolated by Koritschoner (1923) from the brain of a patient who died of an encephalomyelitis six weeks after having been bitten and having undergone a course of antirabies treatment. Rabbits inoculated intracerebrally with a brain emulsion developed an encephalitis with paralytic symptoms after an incubation period of 3 to 5 days, and passage virus was shown to be virulent for mice, guinea-pigs and dogs. Corneal inoculation in rabbits produced encephalitis without any keratitis. Koritschoner concluded that this was an atypical strain of herpes virus, an opinion shared by Luger and Lauda (1924). From their study of this strain Doerr and Zdansky (1924) formed the opinion that it was more nearly related to rabies virus and Nicolau (1925) fully supports this contention. It can be distinguished from herpes virus since:

(1) It is virulent for the dog. The insusceptibility of this animal to herpes virus though not absolute (Remlinger and Bailly, 1926) is of a high order.

(2) It is non-keratogenic in the rabbit.

(3) Rabbits immune to herpes virus are not protected against it. Intracerebral inoculation in the dog produces paralytic symptoms after an incubation period of four days and although Negri bodies cannot be demonstrated in the lesions of the central nervous system, the changes resemble those produced by the virus of rabies. By means of serum-neutralization experiments Takaki (1926) claims to have identified the Koritschoner virus with rabies virus.

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CHAPTER XIV. FOWL PLAGUE.

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General Pathology.

BY JOHN P. RICE.

FOWL PLAGUE is an acute, infectious disease of birds caused by a filterable virus. It is known under a large variety of names, such as exudative typhus of birds, epizootic peritonitis, kyanolophiæa and bird pest. The latter name seems the most suitable as it covers the many species of birds which are susceptible to attack and is non-committal regarding the variable symptoms and lesions of the disease.

History.

Fowl plague was first reported in 1878 in Italy, but does not appear to have spread to other countries till about 12 years later, when it invaded the Tyrol and Germany. Outbreaks have since been recorded in Austria, Hungary, Switzerland, France, Belgium, Holland, England, Egypt, China, Japan, United States of America, Argentine and Brazil. The tendency during the last decade has been for the disease to become less prevalent, but more universally distributed. With the exception of a severe outbreak in the United States of America in 1924, world losses from fowl plague have markedly declined in recent years. Thus, in Italy, where at one time the disease was a veritable scourge, the Director-General of Public Health (1927) reported that it had ceased to constitute a serious danger to flocks. Only three outbreaks of fowl plague are known to have occurred in England, and the disease has never been an important cause of loss to poultry farmers in this country.

Pathogenicity.

The disease attacks only birds, chiefly fowls, turkeys and geese, but many other species are susceptible, including ducks, pigeons, pheasants, guinea-fowl, blackbirds, sparrows, sparrow-hawks, owls and parrots. Generally the disease is confined to one species and does not pass naturally to other species, especially to waterfowl. This rule is by no means invariable, however, as several outbreaks are on record where two or more species have been involved. Thus, Freese (1908) reported the simultaneous occurrence of the disease in fowls and geese and Gerlach and Michalka (1926) in fowls, turkeys, geese and other birds.

Natural Infection.

The route by which the virus of fowl plague enters the body under natural conditions is unknown. Healthy birds usually contract the disease by association with affected birds or by being placed in coops, crates or other poultry equipment previously used for sick birds. It is probable that infection is spread occasionally by diseased wild birds and mechanically by persons. Several writers suggest that rodents may act as mechanical carriers, but the point has not been proved. Mohler (1926) found evidence which indicated that birds may recover and still be carriers of infection. The nature of the evidence has not, however, been disclosed.

Symptoms.

The incubation period under natural conditions varies from 3 to 5 days; occasionally it may extend to 7 days.

The symptoms exhibited by affected fowl are not characteristic, and do not serve to distinguish bird pest from other acute diseases such as fowl cholera. Generally the disease runs a very rapid course, and the bird may be found dead without showing any premonitory symptoms, or signs of illness may be presented for only a few hours. Usually the disease terminates fatally within 24 hours after the appearance of symptoms. Exceptionally the illness may last a week.

Affected birds isolate themselves from the rest of the flock and seek any shade that may be available. They show little or no desire for food and stand or lie in a semi-comatose state with wings and tail drooping, head drawn in and eyes wholly or partly closed. There is usually a certain amount of dyspnœa due to the accumulation of mucus in the upper air passages and nasal cavities. One or both eyes may show a serous or mucus discharge. These catarrhal symptoms appear to have been very common in the American outbreaks reported by Krohn (1925) and others. As Mohler (1926) pointed out, infectious bronchitis was very prevalent in the United States at the same time as fowl plague, and it is probable that many of the cases of fowl plague showing respiratory symptoms were complicated by the former disease. Generally, European observers have not stressed the occurrence of such symptoms. Freese (1908) went so far as to regard them as being of a mechanical nature due to the inability of the bird to expel the normal secretions. There is much to be said for this view, because in many other diseases of birds not affecting the respiratory tract, an abundant quantity of mucus may be found at post-mortem examination in the mouth cavity and throat. False membranes were found in these situations by Maggiora and Valenti (1903), and, in a small proportion of cases, by Krohn (1925). Further investigation of such cases requires to be carried out, however, in order definitely to exclude a concomitant infection with the virus of avian diphtheria.

Another symptom commonly encountered in American outbreaks, and apparently rare in European cases, was œdema of the head and neck, especially of the wattles and submandibular tissue. This symptom, if present, is of diagnostic importance (Krohn, 1925; Beaudette, 1925; Johnson, 1925; Stubbs, 1926). In the later stages of the disease, cyanosis of the comb and skin generally may become very pronounced.

Diarrhœa is not commonly present, although towards the close of the illness excreta may be evacuated in considerable quantities, probably due to relaxation of the sphincter of the vent. Nervous symptoms are always prominent in plague. The bird staggers and finally is unable to stand. It exhibits involuntary movements of the head and neck, varying from a slight twitching to violent spasms. The temperature, which is elevated 3 to 5° F. at the height of the disease, becomes subnormal and death takes place after a short struggle.

Recovery is not uncommon. In the first English outbreak reported by Rice (1922), several birds which were obviously ill recovered. Similar experiences are recorded by Ostertag and Wolffhügel (1902), Freese (1908) and Erdmann (1920).

The disease often runs a slower course in geese and nervous symptoms usually predominate.

The mortality varies within wide limits and ranges from 40 per cent. to 100 per cent. with an average of about 70 per cent.

Pathology.

The essential lesions in fowl plague are linear and punctiform hæmorrhages which may occur in almost any part of the body. Many authors have attributed diagnostic importance to their presence in certain organs, especially the proventriculus. They also commonly occur in the œsophagus, in the subepicardial and gizzard fat and on the inner surface of the sternum.

A considerable literature has grown up regarding the post-mortem appearances in fowl plague, but the findings are so diverse that there seems little point in discussing them at length. Swelling of the kidneys is emphasized by Depperich (1907), Freese (1908), Beaudette (1925) and others. The former worker also attaches importance to congestion of the liver. The value of these observations, however, is seriously open to question, as these organs, even in normal fowl, vary greatly in size and appearance. There also seems ground for criticizing the many statements that catarrh of the upper air passages is usually present. While the majority of authors are in agreement with this view, it would appear that their opinions are based merely on the presence of mucus in these situations and not on a histological study of the parts. Reference has already been made under 'symptoms' to the occurrence of œdema, involving the head and neck and of cyanosis of the skin. Other lesions which may be encountered are enteritis, usually confined to the duodenum, pericardial and peritoneal exudates and congestion of the ovary. To summarize, autopsy may disclose lesions affecting almost every organ in the body, or the findings may be entirely negative, especially in very acute cases.

Inclusion bodies closely resembling those seen in rabies were found in the brains of affected birds, chiefly geese, by Schiffmann (1907) and others. According to Mattel (1926), they may be inside the nerve-cells or lie free in the surrounding tissue. Their diameter is usually about 10μ, but they may be much smaller. They can be demonstrated by the usual methods such as that of Mann. The constancy and significance of these brain inclusions have not yet been fully determined.

Diagnosis.

There is nothing in either the symptoms or lesions which enables a positive diagnosis of fowl plague to be made. Bacteriological examination of the fresh blood fails to reveal the presence of any bacteria. Filtrates through porcelain are virulent for fowl while rabbits are immune. The pigeon, being usually resistant, is often used as a test animal, but it would appear unsuitable as it may succumb to the inoculation of plague virus, as shown by Nakamura and Kawamura (1926) and Miessner and Berge (1926). Diagnosis is greatly facilitated by the use of specific immune serum. Birds which have received serum resist inoculation, whereas birds not so protected readily succumb.

For the diagnosis of the disease in geese and waterfowl generally, animal experimentation should be made with both blood and an emulsion of brain substance.

Differential Diagnosis.

Fowl plague may be confused with a large number of other diseases. It bears a close resemblance to so-called *pseudo-pest* or Newcastle disease which has been described by Doyle (1927) and Picard (1928). The chief distinguishing features are that in pseudo-pest the illness is more protracted, the disease can be readily contracted by contact, and pigeons can be easily infected. Cross-immunity tests may also be employed. *Infectious bronchitis* (Beach, 1926) may be distinguished from plague by the fact that the lesions of the former disease are confined to the trachea. In *fowl cholera* usually several species of poultry are involved. The liver shows characteristic necrotic foci. The causal bacillus may be demonstrated in the blood by microscopical and cultural methods. Rabbits are susceptible to inoculation. *Fowl typhoid* may be differentiated by cultural examination of the tissues for *Bacillus sanguinarium.* Animal inoculation of blood or emulsions of organs usually fails in this disease. In *spirochætosis, apoplectiform septicæmia* and *sleeping sickness of birds,* the causal organisms may be demonstrated by the usual methods. Hæmorrhages in the proventriculus are a constant feature of *phosphorus* poisoning, but they are deeper than in fowl plague. The mucous membrane is usually eroded. The crop and gizzard contents smell strongly of phosphorus.

Treatment and Prevention.

Treatment of the disease is unavailing. The control measures which should be adopted are similar to those employed against other infectious diseases. The isolation of new arrivals for one week is a routine precaution. When the disease is prevalent, an individual examination of the flock should be made at frequent intervals and all birds showing the slightest signs of indisposition should be quarantined. When plague has been confirmed in the flock, affected birds should be destroyed and the carcasses burned or buried deeply. Safe disposal of the droppings, litter, &c., is indicated, together with thorough disinfection of the plant and utensils. The use of dry mash hoppers should be discontinued, grain should be fed in troughs and the drinking water should be replenished daily.

In countries where the disease is not enzootic, the best policy is to slaughter affected flocks and to restock after thorough cleaning and disinfection. It is advisable in the first instance to place only a few birds in the affected premises and to observe them for one week in order to test if infection is absent.

Legislation, requiring notification of the disease, regulation of traffic of poultry and disinfection of railway waggons, poultry crates, &c., may be necessary.

The Experimental Disease.

By Charles Todd.

The experimental disease produced in fowls by the injection of fowl plague material is acutely septicæmic, and, with strains of the virus which have already been passed several times through the fowl, death usually occurs in from 36 to 48 hours, although in some cases it may be delayed until the third or fourth day. The size of the dose appears to have very little influence on the time of death, and birds receiving a million times the minimal infecting dose do not necessarily die sooner than those receiving only a few minimal infecting doses.

About 24 hours after inoculation the birds become somnolent and remain huddled up with raised feathers and closed eyes, oblivious of their surroundings and in a semi-comatose condition ; their temperature rises 1 or 2° C., and there may be a discharge of glairy mucus from the mouth and nostrils. Death takes place without the appearance of any marked nervous symptoms and generally with a subnormal temperature.

The experimental disease as a rule runs a course so acute that there is little time for the development of any marked lesions and examination of the dead bird may show no changes other than those associated with an acute septicæmia, but usually petechial hæmorrhages, particularly noticeable on the surface of the heart and intestines, are observed, and not infrequently there is an increase in the amount of fluid in the pericardial sac, which may be ballooned out with a semi-fluid gelatinous exudate. The liver is generally yellowish in colour and very friable.

Ætiology and Nature of the Virus.

The disease is caused by an ultramicroscopic virus which at the time of death is distributed throughout the body, all parts of which are highly infective.

Filterability. The fact that the causal agent of the disease can pass through both the Berkefeld and Chamberland filters was shown by Centanni and Savonuzzi (1901). At that date only four ' filter-passing ' viruses were known (viz. foot-and-mouth disease, bovine pleuro-pneumonia, tobacco mosaic and horse sickness) and the discovery of a fifth attracted much attention, particularly as fowl plague presented peculiar advantages for experimental investigation. Centanni and Savonuzzi's results were confirmed by a number of workers, notably Lode and Gruber (1901), Ostertag and Wolffhügel (1902), Künnemann (1902), Maggiora and Valenti (1903), Rosenthal (1908) and Andriewsky (1915), the general experience being that the virus always passed the Berkefeld candle and generally, although not always, the Chamberland. Rosenthal (1908), as the result of a large number of experiments, came to the conclusion that no porous filter could be relied upon to stop the virus with certainty, although certain filters would not let through more than one ten-thousandth part of the virus present in the original fluid. Andriewsky (1915) working with Bechold's graded ultrafilters, prepared by dissolving different percentages of guncotton in glacial acetic acid, found that the virus passed a filter which did not allow the passage of hæmoglobin, and hence concluded that the virus must be smaller than the molecules of hæmoglobin, and, therefore, could not be in the form of cells resembling any known animal or vegetable cells. In this connection, however, it has been pointed out (Schweizer, 1921) that much smaller amounts of fowl plague virus than of hæmoglobin can probably be detected owing to the extraordinary susceptibility of the fowl to the virus. Miyaji (1914) could not confirm the passage of the virus through the ultrafilter, although he believed that its size approaches the molecular dimensions of proteins.

The virus, as it exists in the clear cell-free blood-serum, is not thrown down in the centrifuge at ordinary speeds, but Landsteiner and Russ (Landsteiner, 1906) using velocities of 4,000 to 6,000 revolutions per minute for varying periods up to 11 hours, believed that they obtained definite evidence that the upper layers became less virulent on prolonged centrifuging.

Some experiments bearing on the nature of the virus of fowl plague have recently been carried out by Kempner (1927). Fowls which had been infected with fowl plague were bled when dying from the disease, and their blood heparinized and centrifuged. The cell-free plasma after being controlled for sterility and virulence was manometrically examined. It was found that while the plasma of normal birds does not ' breath ', the plasma of infected birds does so—6 c.cm. of virulent plasma at 42° C. consuming as much oxygen as 3,000 million red blood corpuscles of the fowl. The amount of oxygen consumed by 6 c.cm. of the virulent plasma was 29 c.mm. during the first hour of the test and 20 c.mm. during the second.

The sera of 50 infected fowls were examined and ' breathing ' could be demonstrated in the case of all those which were highly virulent. The respiration of fowl plague plasma was much more resistant to the action of hydrocyanic acid and of octyl alcohol than was the respiration of cells or bacteria, and by saturation of the virulent blood with octyl alcohol, the cell respiration could be entirely stopped while the virus respiration continued. These results are interesting, but the work is recent and apparently has not yet been confirmed.

Action of Physical and Chemical Agents on the Virus.

Drying. The resistance of the virus to drying depends very markedly on the degree to which this is carried out, but if the drying is quickly and thoroughly done the virus shows itself highly resistant to desiccation. The data given in the literature mostly refer to experiments in which virulent blood or thin slices of the tissues have been dried by simple exposure to the air and then kept in the dark at room temperature. Under these circumstances the infectivity disappears in from 2 to 7 weeks (Maggiora and Valenti, 1903 ; Centanni, 1902 ; Lode, 1902 ; Maue, 1904). Virulent blood carefully dried *in vacuo* over phosphoric anhydride and then kept in the dark in sealed glass tubes at 20° C., retains its infectivity for considerably over a year (417 days), and Maue (1904) found that the spinal cord dried over calcium chloride and kept in the desiccator at 20° C. was still infective after 233 days.

Temperature. At room temperature virulent blood, free from bacterial contamination, when kept sealed up in a glass tube is still infective after 3 weeks (Centanni, 1902), but the infectivity is maintained much longer at lower temperatures, and when the blood was kept in sealed capillaries in the ice-safe at 8° C. it was fully active after 144 days (Maue, 1904). For experimental purposes the cell-free serum or citrated blood-plasma is best kept in a frozen condition at —10° C. ; under these conditions it maintains its infectivity practically unchanged for some months, and no appreciable change in the minimal infective dose is observed after 3 months' storage at this temperature.

The virus as it exists in the brain or in whole blood is inactivated by heating for an hour at 55° C., or for 3 days at 47° C. (Jouan and Staub, 1920).

Chemical agents. The virus is very resistant to the action of glycerol, and pieces of tissue kept in 50 per cent. glycerol retain their infectivity for 3 to 5 months if not bacterially contaminated (Gerlach and Michalka, 1926). Ether has very little action and a mixture of one part of ether with four parts of virulent blood kept in a closed vessel was found to be still infective after three days in one experiment and 15 days in another (Centanni, 1902). Formaldehyde inactivates the virus with some ease, a 20 per cent. emulsion of the infective organs in a 0·2 per cent. dilution of formalin in saline being non-virulent after 48 hours at 37° C. (Staub, 1926). The virus is not very resistant to chloroform, and a current of air bubbled through chloroform and then through an emulsion of fowl plague organs for 1¼ hours rendered the virus inactive, and a similar result was obtained by shaking the emulsion with 10 per cent. of its volume of toluol and allowing the mixture to stand for 18 hours (Lode 1907).

Bacterial growth is very prejudicial to the virus and even the presence of glycerol is not sufficient to prevent contaminating organisms from bringing about its inactivation.

Artificial Culture of the Virus.

All attempts to grow the virus in artificial media have given quite negative results, but Marchoux (1908) reported the successful cultivation of the virus in defibrinated normal fowl-blood. The cultures were made in test-tubes of 20 mm. diameter containing a column of glucose peptone agar, and were grown aerobically. Inoculation of the tubes with virulent blood was carried out by means of a fine platinum wire, so as to use a minimum of the inoculum, and the cultures grown for three days. In this way a series of 10 successive passages was made. Marchoux's work was confirmed by Landsteiner and Berliner (1913), who carried out 9 consecutive passages of the virus in this medium, each passage representing a dilution of 1 in 200. After 9 passages the virulence of the inoculated medium was the same as that of the original blood. Cultures made in ascitic fluid, fowl-serum, rabbit's blood, and fowl's blood laked by freezing were all negative and the authors conclude that the presence of intact red-blood corpuscles is probably necessary. Todd (1928[1]) repeated this work, using an English strain of the virus and the technique given by Marchoux, but was unable to obtain any evidence of multiplication of the virus. Jouan and Staub (1920) found that the virus could be propagated in the developing egg of the fowl, but that development must have gone on for at least three days before the eggs were used.

Susceptibility to Experimental Infection.

No animals other than birds can be infected with the virus. The domestic fowl, turkey and pheasant are very susceptible, as are a number of wild-birds, e.g. the sparrow, blackbird, starling, goldfinch, sparrow-hawk, &c. The virus reaches a very high degree of virulence when passed several times through fowls. Thus, Gerlach and Michalka (1926) found that a strain which originally killed fowls in 7 days, did so in from 36 to 48 hours after being passed 4 times. The adult pigeon as a rule is not susceptible, but young pigeons can be infected, although the results are very irregular. In Japan, the pigeon appears to be more susceptible than in Europe (Nakamura and Kawamura, 1926). Generally speaking, waterfowl as a class are insusceptible.

It is an interesting fact that, while several naturally occurring outbreaks of fowl plague have been observed amongst geese (Freese, 1908; Kraus and Loewy, 1915; Miessner and Berge, 1926), and strains of the virus have been found which infect the goose (Kraus and Loewy, 1915), the adult goose is normally quite insusceptible to subcutaneous or intramuscular injection of the majority of strains of fowl plague, although it can be infected intracerebrally. Young geese can usually be infected by intramuscular injection, but in them the experimental disease is much less acute than in the fowl, and is characterized by marked nervous symptoms, as is also the case in the pigeon. Kleine (1905) holds that these nervous symptoms are not due to an intoxication, but to the localization of the virus in the central nervous system, and this view is supported by the fact that the brain of the dead goose is very highly virulent, whilst the blood at the time of death may no longer infect.

Kraus and Loewy (1915) investigated the varying infectivity of different strains of fowl plague and pointed out the resemblance of their behaviour to that of different strains of rabies.

Presence of the Virus in the Tissues and Body Fluids.

In fowls dead of the acute disease all the tissues and fluids of the body are very highly infective. The blood at the time of death commonly kills at a dose of 1/1,000,000 c.cm., and sometimes at a much smaller amount. Krumwiede, Gerber and Provost (1925) were able to infect fowls with a dose of only 1/1,000,000,000 c.cm. In the centrifuged blood the virus is found mainly in the white cell layer, its concentration there being roughly one hundred times that found in the clear plasma or in the washed red blood corpuscles (Todd, 1928[1]). The bile, nasal and cloacal secretions and fæces are all infective.

Methods of Infection.

Fowls are infected with certainty by subcutaneous, intramuscular, or intravenous injection of very small doses of virulent material, and the virus passes through the uninjured mucous membranes with ease, so that it is only necessary to drop the infective material into the conjunctival sac or cloaca in order to produce infection. The results of feeding are irregular and Doerr and Zdansky (1924) only succeeded in 6 cases out of 40 (i.e. 15 per cent.). Under laboratory conditions contact infection is rare, and most observers report negative results, but Doyle (1927) made the interesting observation that while a healthy fowl does not contract fowl plague when placed in a cage with an infected bird, the disease invariably results if the intensity of the infection is increased by the employment of 3 or 4 infected birds.

The Production of Active Immunity in the Fowl.

In countries which have been free from outbreaks of fowl plague for some time, the domestic fowl is very highly susceptible, and artificially infected birds invariably die of the disease. The naturally occurring disease, on the other hand, is not always fatal, and a certain number of fowls, particularly young birds, may recover and subsequently show an absolute immunity (Ostertag and Wolffhügel, 1902; Erdmann, 1920).

The knowledge that the fowl is capable of naturally acquiring such a high degree of immunity has led to numerous attempts to produce this immunity by artificial means; but although the problem was attacked by a number of workers, the results were for many years most disappointing. Attempts to immunize fowls with heated virulent materials (blood and organs) made by Maggiora and Valenti (1903), Maue (1904), Russ (1906), v. Prowazek (1908) and others were uniformly unsuccessful, and in 1907, Kraus and Schiffmann stated that a review of the literature showed that at that date no worker had been able either to produce immunity in susceptible animals or to demonstrate the presence of antibodies, with the possible exception of Maggiora and Valenti (1904), who claimed to have produced a protective serum by inoculation of the adult goose, which is naturally immune to the virus except when inoculated intracerebrally.

All attempts to produce an active immunity by the injection of the virus modified by the action of heat, light, or other agents having given entirely negative results, Kraus and Schiffmann (1907), influenced by the resemblance of the behaviour of the virus of fowl plague to that of rabies, found that it was possible to immunize young geese, which are less susceptible than fowls, by the injection of the dried spinal cord of infected young geese. Later, Kraus and Loewy (1915) were able to produce immunity in fowls by the same method.

In 1920, Jouan and Staub described the successful vaccination of fowls with heated virulent blood. These workers used virulent blood which had been heated for 3 days at 46 to 47° C., or for three-quarters of an hour at 60° C., and obtained equally good results in either case. The heated blood was injected intramuscularly, and one injection was sufficient to produce a solid immunity, but it was necessary to use a comparatively large volume of the heated blood—not less than 10 c.cm.

In view of the quite unanimous experience of all previous workers, that immunity could not be produced by the injection of heated virulent materials—whether blood or organs—Jouan and Staub's results are very striking. It is true that these workers gave a large dose of the virulent blood, but immunity was produced after a single dose, whereas all other workers have failed to obtain immunity even after repeated injections of the heated virus, and since the publication of Jouan and Staub's work, Doyle (1927), who treated 4 fowls with a series of 6 injections of 1 c.cm. of an emulsion of the spleen of infected fowls—this emulsion having been heated at 60° C. for an hour—did not obtain immunity in any of the birds. Gerlach and Michalka (1926) and Todd (1928[2]) also were unable to immunize birds with large and repeated doses of the dead virus.

In 1926, Staub reported that he was able to vaccinate fowls by means of injections of a formalinized saline emulsion of the spleen of infected birds, this vaccine being prepared by emulsifying 1 gm. of the spleen with 5 c.cm. of a 1 in 500 solution of formalin in saline, and keeping the mixture at 37° C. for 48 hours. A single dose of the vaccine was sufficient to immunize a fowl. Doyle (1927) repeated these experiments with formalinized emulsions, but was unable to obtain immunity against a subsequent dose of virulent blood, even 6 progressively increasing doses of the vaccine—from 1 c.cm. to 5 c.cm.—given at 5-day intervals not producing immunity. Todd (1928[2]) also failed to produce immunity by repeated injections of the formalinized organs, but found that a solid immunity could be produced in fowls by the use of a phenol glycerin vaccine prepared from the livers of birds dead of the disease. In order to ensure immunity three doses of the vaccine, given at intervals of a week, were necessary. An equally satisfactory vaccine could be prepared by the use of tricresol in place of phenol. Fowls immunized with a vaccine prepared from the English strain of the virus were found to be immune against the Dutch (de Bliech), the French (Staub), and the German (Miessner—goose) viruses.

Immune Serum.

Maggiora and Valenti (1904) stated that geese treated with the virus yielded a serum which in a dose of 5 c.cm. 'had a certain preventive action in most of the experiments' on fowls and pheasants, but they gave no details. Kraus and Loewy (1915) found that 5 c.cm. of the serum of immunized geese, protected fowls against a dose of the virus given 24 hours later. Jouan and Staub (1920) used fowls which had been immunized either by heated vaccine or sero-vaccination, and reinforced their immunity by the intravenous injection of 25 to 40 c.cm. of virulent blood, given in two doses. Ten days after the last injection the serum of these birds was highly protective: an intravenous injection of 0·25 to 1·0 c.cm. protecting fowls against an intramuscular injection of 1/16,000 c.cm. of virulent blood given 24 hours later. In their experience the goose did not yield a good serum. Todd (1928[3]) by the hyperimmunization of fowls previously vaccinated with phenol glycerin vaccine was also able to produce an efficient protective serum.

Animals insusceptible to the virus appear to be incapable of producing an immune serum and no protective action could be demonstrated in the sera of the sheep, goat, ass, duck, goose or pigeon treated with the virus (Maue, 1904).

The serum of hyperimmunized fowls has a powerful protective action either when mixed with the virus before injection or when the serum and virus are given separately by the same or different routes. Thus, in the case of a particular serum tested it was found that a mixture of 1 c.cm. of the serum with 40,000 minimal infecting doses of the virus was non-infective on intramuscular injection, but that the injection gave rise to no immunity. When the serum and virus were given simultaneously, but on different sides of the body 5 c.cm. of the same serum protected against 10,000 minimal infective doses of the virus and the fowl was subsequently found to be solidly immune.

While the immunization of flocks by means of the serum is obviously not practicable—either on economic or other grounds—the discovery of a specific immune serum has rendered the diagnosis of fowl plague comparatively simple, and this should clear up much of the uncertainty as to the exact nature of the outbreaks of acute septicæmic disease met with in different species of birds.

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Plague of Blackbirds.

By Charles Todd.

This is a highly fatal septicæmic disease, due to an ultramicroscopic virus which is capable of passing the Berkefeld filter, affecting mainly the blackbird and mistle-thrush. Attention was first called to the disease in the autumn of 1901 by the occurrence of a striking mortality amongst birds of the thrush family in the neighbourhood of Modena. The dead birds found were chiefly blackbirds and mistle-thrushes with some fieldfares and other birds. Dead starlings were also seen but in smaller numbers.

The disease was investigated by Maggiora and Valenti (1903), who were able to infect the blackbird, kestrel, an owl (*Strix bubo*) and sometimes the sparrow, but not the fowl or pigeon. Rabbits and mice were insusceptible. Susceptible birds died in 2 to 3 days. The lesions observed *post mortem* were not characteristic, but were not unlike those met with in birds dead of fowl plague. Microscopical observation showed no bacterial or other organisms, and cultures from the blood and organs were sterile. The blood and organs were highly infective and the virus was found to traverse a Berkefeld candle controlled for its impermeability to ordinary bacteria.

As a severe outbreak of fowl plague had occurred in the district earlier in the same year, the possibility that the disease was a modified form of fowl plague was naturally considered, but Maggiora and Valenti regarded this as improbable, particularly in view of the complete insusceptibility of the fowl to the new virus and of the rapid loss of virulence shown by the virus on keeping. They were, therefore, of the opinion that the disease must be considered as being due to a specific and hitherto unrecognized virus. Unfortunately at the time when this work was done, immunity to fowl plague could not be artificially produced, so that neither cross-immunity tests nor experiments on the effect of fowl plague immune serum could be made.

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CHAPTER XV. DOG DISTEMPER.

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Introduction.

Distemper of dogs is a disease which has been recognized for many years, in this country (Jenner, 1809; Delabere Blaine, 1817), as the most serious disorder to which young dogs are liable. The severity of the illness may vary from case to case within wide limits, and the symptoms displayed in different cases may be of very diverse character. The great divergence between cases led to the differentiation of several types of the disorder, and, at one time, it was thought possible that there might be more than one distinct disease included under the term dog distemper. It now seems clear, however, that there is a single infective agent common to the different types, and that many of the symptoms at one time regarded as part and parcel of the distemper syndrome are really due to invasion of damaged tissue by secondary infective agents. The evidence in favour of this view is based on the experimental study of the disease in susceptible puppies. By forcing the disease upon a perfectly healthy animal, with minimal amounts of infective material, as free from contaminants as is possible and transmitting the disease from dog to dog, the secondary infective agents can be weeded out and dog distemper in its simplest form is left for study. The principal difficulty in work of this kind is an adequate supply of susceptible animals. Distemper infection is so prevalent in the canine population that many young dogs contract the disease at an early age, and as all survivors are immune, it follows that a fair proportion of young dogs obtained in a haphazard manner will be useless for experiment. Carré (1905) employed quite young puppies where the chances of encountering an immune animal were small. Dunkin and Laidlaw (1926[1 & 2]; Laidlaw and Dunkin, 1926, 1928[1, 2 & 3]) had the inestimable advantage of a stock of dogs bred under the strictest possible isolation, and they found that every puppy from this source was susceptible to the disease.

Experimental Distemper in the Dog.

Experimental distemper in the dog may be defined briefly as follows: it is an acute infectious fever characterized by an incubation period of four days, a coryza at the onset of the disease, an unusual temperature curve, severe gastro-intestinal disturbance and a variable set of symptoms due to inflammation in the respiratory tract. In a proportion of cases nerve symptoms caused by an encephalitis may develop.

The onset of illness is sharply defined by fever and a watery discharge from the eyes and nose. The discharge is frequently trivial, even in very severe cases, but it may be profuse. Within 24 hours it may become faintly purulent and the conjunctivæ acutely congested. Crusts, composed of half-dried cells, collect at the angles of the eyelids. The temperature rises abruptly at the commencement of the illness and may reach 105° F., or even more, within 24 hours. After one or two days it subsides again to near the normal level of 101·4° F., but it soon rises again. The second rise is slower than the first and much more prolonged. Its duration is exceedingly variable, and may be as short as 48 hours in the milder cases, or more than three weeks in the more severe. At the onset of the illness the dog usually vomits and refuses all food. With the fall of temperature appetite returns for a time, to diminish once more as the secondary fever develops. In practically every case there is diarrhœa, and in the secondary febrile period this may be profuse. The motions are slimy, may contain streaks of blood, and have an offensive and somewhat characteristic odour. The diarrhœa may persist for some days after the remission of the fever. The poor appetite coupled with the diarrhœa interferes with nutrition, and the animal wastes rapidly.

Symptoms from the respiratory system are usually slight. Some cough is common in the period of the secondary fever, but definite bronchitis or broncho-pneumonia does not occur. At the same time there is nearly always some evidence of inflammation in the respiratory passages during life or at autopsy. Definite involvement of the nervous system may take place early or late in the disease, and is of very variable occurrence. The earliest symptom is a sudden attack of semi-consciousness with vigorous chewing movements, accompanied by the secretion of ropy saliva which exudes from the angles of the jaws. The fit may pass off after a few seconds or persist for over a minute, after which the dog appears to be perfectly conscious once more. Fits of this type may occur at irregular intervals for some days, but, as a rule, they become more frequent and severe, and merge into attacks of violent epileptiform convulsions. Muscular spasms and twitches may be observed in those cases which develop nerve symptoms late in the disease, and these in turn may become exaggerated and lead to generalized convulsions.

Distemper as seen in Veterinary Practice.

Dog distemper, as it is seen in veterinary practice, for example in kennels of foxhounds, usually shows additional features, and some divergencies. The incubation period of the natural disease is stated to be more variable and usually more prolonged. Well-informed veterinary opinion puts the maximum period at three weeks. Some increase of the incubation period under natural, as contrasted with the experimental, conditions is only to be expected (cf. measles), but it is difficult to account for the very long incubation period as observed clinically, unless it be supposed that the clinical diagnosis is only made in the second febrile period when the symptoms are much more characteristic.

In epizootics in kennels it is usual for many cases to show striking suppurative catarrhal symptoms. The discharges from the eyes and nose frequently become purulent, the eyelids may be stuck together and the nose obstructed. Superficial ulcers in the mouth may develop. Many cases of definite bronchitis with a husky cough may be observed, and for some observers this is still regarded as an essential feature of the disease. When cases with bronchitis occur it is highly probable that sooner or later broncho-pneumonia will appear and some of these will pass into cases of gangrene of the lung or pyothorax. Vesico-pustules on the abdomen were for long regarded as a classical symptom of the disease, but they have been shown to be of no special significance (Dunkin, 1926), yet it is a fact that they are sometimes very common in distemper cases. In some outbreaks of distemper diarrhœa may become a very prominent symptom and may prove very exhausting to the patient. In other outbreaks the proportion of cases which develop fits, muscle twitches or convulsions is very high, and there is then a high mortality rate, and even if recovery occurs there is frequently some permanent damage to the nervous system remaining.

Mortality.

In the case of the experimental disease the mortality rate is not great even in cases which are very severe, provided that the nervous system escapes infection, and complications do not arise. There are cases, particularly in young puppies, where the infection overwhelms the patient and sudden collapse develops during the second febrile period, and the animal then dies within a few hours with a subnormal temperature. There are other cases where nervous symptoms supervene in an animal which appears to be recovering; but on the whole the experimental disease contrasts favourably with the epizootic, where bronchitis and pneumonia bring death to patients which might otherwise have recovered. Epizootic distemper is analogous to epidemic influenza where the secondary invasion of the lungs by various types of bacteria is directly responsible for many fatalities.

Occurrence and Spread of Distemper.

Dog distemper is a disease which occurs sporadically in every country where a canine population exists. At times outbreaks of a virulent nature pass over a country with very serious results to the canine inhabitants. In Greenland, for example, there have been three violent epizootics (1888, 1896, 1904) which took severe toll of the dogs in the country (Hjortlund, 1908). Puppies before they are weaned appear to be resistant to infection, but thereafter they become susceptible, and, as a rule, it is the younger animals that suffer most severely, though the older animals are not immune if they have been isolated from, or escaped, infection in their youth. It thus comes about that in every foxhound kennel there is an annual outbreak of distemper shortly after the return of the puppies from 'walk', and not infrequently 50 to 70 per cent. of the young entry may perish.

The contagium has never been identified, but observation shows that it is spread by contact between the sick and susceptible, and it is exceedingly probable that it may be air-borne over short distances in confined spaces (Dunkin and Laidlaw, 1926). It has not been proved that carriers exist, though there is some evidence that a recovered animal may be infective for a time. There is no evidence that ectoparasites act as vectors of the disease. The contagium is readily destroyed and can only be preserved with difficulty outside the body, so that the initiation of epizootics is often difficult to explain.

Morbid Anatomy.

There are no characteristic appearances to be found at autopsy following death from the experimental disease. The body is usually wasted and the tissues drier than normal. The lymphatic apparatus of the alimentary canal is inflamed. The Peyer's patches are enlarged, swollen, and sometimes very prominent. The abdominal mesenteric glands are enlarged and exude much milky fluid on section. Scattered red points may be seen on the mucosa of the small and large intestine, but definite ulcers do not occur. The spleen is paler than normal and slightly enlarged. In rare instances the liver may be jaundiced, but as a rule it appears normal. There may be excess of fluid in the pericardium and small red areas in the lungs. Broncho-pneumonia does not occur. The bronchial tubes may be congested, but a purulent exudate is quite uncommon. The brain usually appears to be quite normal. In cases which showed acute nerve symptoms during life the meninges are congested, the ventricles are dilated and there is excess of cerebrospinal fluid.

Histological examination again shows nothing characteristic. The endothelial cells of the lymphatic system, Peyer's patches, abdominal lymph glands and spleen show signs of activity. These cells are more numerous than usual, and mitoses are common; a certain number of cells show degenerative changes, and others contain eosinophile masses of irregular shape in the protoplasm. Remnants of phagocytosed red and white cells may be detected. Lymph glands and spleen are infective for susceptible animals in doses as low as 0·000,01 gm.

A number of observers have described inclusion bodies which were named ' negria canis ' and regarded as characteristic by Sinigaglia (1913). In the experimental disease inclusion bodies, whatever their precise nature may ultimately be shown to be, do not occur with that regularity as to numbers, size and disposition which is to be expected if they are directly associated with the infecting agent.

In cases with acute nerve symptoms there is again very little to be seen by microscopic examination. There is excess of fluid in the spaces in the meninges with a very sparse distribution of mononuclear cells. These are occasionally collected in small groups, and sometimes focal aggregations may be seen in the brain substance. Perivascular cuffing does not occur. In the more acute cases multiple hæmorrhages of varying size may be found in the brain or in the meninges. It should be noted that the brain and the cerebrospinal fluid are bacteriologically sterile, and still infective, often in quite small doses, for susceptible animals.

Lewy and Kantorowicz (1923) described intracellular structures, which they regarded as protozoan parasites, occurring in the brains of dogs which had suffered from the nervous type of ' street ' distemper. In their opinion these resembled the ' parasite ' (named *Encephalitozoon cuniculi* by Levaditi) which may be found in the brains of rabbits infected with the Kling virus of encephalitis lethargica. Evidence was adduced by Lewy, Fraenkel and Kantorowicz (1925) to show that the passage of the Kling virus in dogs resulted in the establishment of dog distemper. The suggestion was obvious that the two diseases were really one and the same. This suggestion can no longer be maintained, as Lewy, Fraenkel and Kuttner (1926) have shown that their distemper virus is filterable and the Kling virus is not. Moreover, it is clear that nerve distemper can exist in the absence of the ' parasite '.

Other observers have described still different pictures in cases of nerve distemper, but the real significance of these it is impossible to define. It is the custom to attribute all lesions of the nervous system in dogs—except rabies—to distemper infection, and this cannot represent the facts. Pugh (1926) drew attention to a clinical type of nerve case with lethargic symptoms, and showed that extensive perivascular cuffing was to be found around the cerebral vessels in this group of cases. He stressed the analogy with encephalitis lethargica in man, though he did not regard the human and canine diseases as identical. The relationship of the condition described by Pugh to distemper remains uncertain, as cases occur in dogs which are ' over distemper ' and which should therefore be immune. Moreover, brain tissue from such cases will not infect ferrets (author's unpublished observation). Further work is required on this subject, and also on the lesions which sometimes follow distemper and cause chronic palsies and transverse myelitis.

The post-mortem appearances in ferrets are very similar to those found in dogs, but multiple small abscesses are almost constantly found in the lips, and occasionally on the abdomen.

In the case of the natural disease in the dog numerous complicating factors are frequently encountered ; for example, purulent bronchitis, broncho-pneumonia, lung gangrene and pyothorax. Vesico-pustules on the abdomen may occur and cancrum oris. Jaundice is rare and intussusception is occasionally met with.

Reviewing the whole position, it is clear that it may be impossible to tell, even after a careful autopsy, whether an animal has died from distemper or not. In the absence of any cultural method for identification of the infecting agent valuable information may usually be gained by inoculating pieces of spleen, lymph glands or brain (in nerve-type cases) into ferrets.

Animals Susceptible.

Dog distemper is a disease which can be transmitted to comparatively few animal species. Man is fortunately insusceptible. Rabbits, guinea-pigs and white rats cannot be infected. Laosson (1882) transmitted the disease to two captive foxes, and Green (1925, 1926) regards distemper in the silver fox as genuine dog distemper, though he has not adduced conclusive evidence in favour of this view. Fox (1923) records the appearance of distemper in wolves, wild dogs, lynxes and racoons, in captivity. The cat was for long regarded as a susceptible animal (Laosson, 1882 ; Krajewski, 1882 ; and others), but, though it undoubtedly suffers from a similar disease, it would seem that this is not identical with that of the dog. Laidlaw and Dunkin (1928) failed to transmit dog distemper to kittens, and also failed to transmit cat distemper to ferrets. Ferrets, stoats and weasels are all susceptible, and ferrets were used extensively by Dunkin and Laidlaw (1926, 1928) in their studies in distemper. The ferret is extremely susceptible, and when infected *very* rarely recovers. In the case of the ferret there is an incubation period of about ten days, after which the eyes and nose show a watery discharge. These may both become purulent, and vesicles appear around the lips, which subsequently become small abscesses. The ferret refuses all food, remains curled up in a corner, wastes steadily, and dies of inanition, or, in a small proportion of cases, develops epileptiform convulsions which may lead to death from asphyxia or aspiration pneumonia. The hedgehog was stated by Sanfelice (1915) to be susceptible to intracerebral inoculation, but Laidlaw and Dunkin (1928) did not find it susceptible to subcutaneous inoculation of the virus.

The Contagium.

The infective agent in dog distemper must now be regarded as a virus or ultramicroscopic organism. This view, originally propounded by Carré (1905), was adversely criticized by many workers. Carré pointed out first, that the disease could be transmitted by small quantities of blood or pericardial fluid, and from neither of these could bacterial growth be secured ; secondly, that the disease could be transmitted by filtrates of body fluids or diluted nasal secretions, and that the filtrates appeared to be bacteriologically sterile. Kregenow (1909) in a series of carefully conducted experiments (using several types of filter and adequate controls in every instance) failed to confirm Carré's findings. Many workers were loath to accept a dual pathology for the disease, and were greatly impressed with the frequency and ease with which bacteria could be cultivated from distemper cases. In 1911, M'Gowan and, independently, Ferry, isolated and described a bacillus which is now named *Bacillus bronchisepticus*, and which is probably identical with that isolated by Copeman in 1900. In 1913, Torrey and Rahe secured the same organism, and all these workers regarded this bacillus as the true infective agent. In 1914, Ferry showed that *B. bronchisepticus* was sufficiently small to pass in small numbers through the pores of standard Berkefeld filters, and he put forward a plea for the more general adoption of standard conditions in all experiments on filtration of infective material. Since this organism was first thrust into prominence it has been isolated with such frequency in distemper cases from the respiratory system that there can be no doubt that it plays some part in the pathology of ' street ' distemper, but that it was the primary infective agent was never established.

In the absence of a supply of dogs of known susceptibility crucial experiments are almost impossible, and the great infectivity of the disease makes it absolutely necessary to work under the most stringent conditions. Every worker on the subject has realized this. M'Gowan considered accurate experimental work impossible. Ferry, Torrey and Rahe knew that it was very difficult, but did the best they could under the circumstances, and came to the conclusion that this bacillus could cause distemper. It is noteworthy that none of these workers found *B. bronchisepticus* in the circulating blood in distemper cases (except as a rare phenomenon), and blood at the height of the first febrile reaction was found by Carré to be the material of choice for transmission experiments. In 1923–4 Puntoni showed that it was possible to passage distemper from dog to dog by intracerebral inoculation, and the infective brain appeared to be bacteriologically sterile. Dunkin and Laidlaw (1926), as the result of a long series of experiments on specially bred dogs and ferrets, showed that dog distemper can be transmitted from dog to dog, from ferret to ferret, or from ferret to dog, by material in which no bacteria could be demonstrated, and which yielded no growth of organisms in ordinary culture media. They showed further that the infective agent could be filtered through bacterial filters of standard type and of proven quality. Their original filtration experiments were all failures, and it is clear that the infective agent will not traverse filters as easily as many other viruses, such as, for example, that of foot-and-mouth disease. The infecting principle, however, was shown to pass through Pasteur Chamberland L2 filters, Mandler filters and membrane filters standardized according to Dr. Kratz of Göttingen. With each of these types of filter visible bacteria were retained. They showed further that the infecting agent could not be cultivated in any straightforward manner.

We have then the following facts: The infective agent in dog distemper cannot be identified under the microscope, cannot be cultivated like ordinary bacteria, will only multiply in the living body of a susceptible animal, and will pass through standard bacterial filters. From which facts it is clear that the contagium of dog distemper belongs to the class of filter-passing viruses.

Properties of the Virus.

The virus of dog distemper is readily destroyed by heat. Laidlaw and Dunkin (1926) found that 55° C. for half an hour did not kill it with certainty, but that one hour at 55° C. or half an hour at 60° C. was adequate for its destruction. On the other hand, cold is the only satisfactory method of maintaining virus in an active form outside the body. At 0° C. the virus will survive for two or three weeks, undergoing slow deterioration all the time, and at, or below, $-10°$ C. the virus will survive for months. It is interesting in this connection to note that in Greenland, where the dog is of supreme economic importance, the law forbids dogs coming from infected districts to use the main sleigh routes. Sad experience has taught the inhabitants that the virus will survive on the frozen sleigh highways for a long time.

Phenol, 0·5 per cent., formaldehyde, 0·1 per cent., kill the virus in the course of a few hours. Lysol, 1 per cent., destroys it quickly. Hexyl resorcinol, 0·1 per cent., kills it in 24 hours. Some of the complex organic urotropines have a decided viricidal action, but urotropine itself appears to be inert.

The virus will survive drying for a time, but deteriorates within two weeks when kept over sulphuric acid. Over phosphorus pentoxide it will remain potent for some weeks.

The disease is readily transmitted to dogs or ferrets by intradermal, subcutaneous, or intracerebral inoculation of infective material. Feeding experiments are uncertain and a ferret may remain unaffected after chewing and swallowing tissue containing some millions of infective doses as measured by the subcutaneous injection method. The insufflation of virus intranasally to dogs will induce disease, and from indirect evidence it seems clear that air-borne infection may occur (Dunkin and Laidlaw, 1926). The virus of distemper cannot be cultivated on any of the ordinary bacteriological culture media. Dunkin and Laidlaw could obtain no evidence of multiplication on any of the media tested, whether with sugars or without, whether under aerobic or anaerobic conditions or with partial tensions of oxygen. Whether the medium was enriched with serum or not, whether the medium contained sterile tissue or blood, or was composed wholly of canine constituents the results appeared to be the same—a gradual disappearance of infectivity: all the subcultures tested proved to be harmless, neither did they induce immunity in the recipients. Tissue culture has not yet been properly tested.

Immunity.

In susceptible races of animals natural immunity probably does not occur. It is true that examples might be quoted of dogs which are said never to have suffered from distemper, and which yet appear to be immune. It is, however, impossible to exclude the possibility, which when all the evidence is examined becomes the probability, that these animals have suffered a mild attack of the disease and recovered; and one attack of distemper is followed, in the great majority of instances, by immunity to further attacks of the disease. There are admittedly well-authenticated instances of multiple attacks in particular cases, but these are quite exceptional, and it is agreed that one attack, as a rule, confers immunity for life. This belief is attested by the fact that a dog which can be guaranteed to be ' over distemper ' has an enhanced value.

This striking immunity in the recovered animal has led every research worker to attempt the immunization of dogs as soon as he was convinced that he had discovered the cause of the disease. Copeman (1900) gave evidence that vaccines of his organism were of value in preventing the disease. Phisalix and Lignières independently isolated what appears to have been the same organism (1903) and vaccines of this bacillus were stated to be beneficial. The Phisalix vaccine was investigated by a Committee in this country and the report was unfavourable (1904). *Bacillus bronchisepticus* is of particular interest as this organism was isolated independently in widely separated localities by M'Gowan (1911), Ferry (1911), and Torrey and Rahe (1913). Vaccines of this bacillus have been very extensively used for the treatment and prevention of distemper infection. The original results appeared to be most encouraging, but as time passed and fuller experience was gained disappointment arose. Hardenbergh (1925) in a recent analysis of a series of cases—over 490 vaccinated animals and 175 controls—failed to find that vaccines of this organism conferred protection against the disease. Other instances might be quoted, and it is now clear that *B. bronchisepticus* vaccines (either living or dead) do not induce a resistant state at all comparable with that which follows recovery from the natural disease.

The experimental study of distemper demonstrated that the visible bacteria which have been isolated from time to time are not the cause of the acute infectious fever which is the foundation of all cases of true distemper. The failure of bacterial vaccines to protect against the disease is thus not surprising, but it must be remembered that invasion of the tissues by visible bacteria does occur in many cases of distemper under field conditions. These are properly regarded as secondary invaders of damaged tissue, but, for some workers, symptoms which are directly related to this secondary invasion are an essential part of the distemper syndrome. Thus, for some workers, suppurative catarrhal symptoms, for others, definite bronchitis with a husky cough, are indispensable symptoms in all cases of genuine distemper. Workers who adopt this standpoint may show to their satisfaction that vaccines may be beneficial in that they eliminate or modify a particular symptom, yet the fundamental infectious fever remains unaffected.

It seems clear that *B. bronchisepticus* can be the cause of an infection without the assistance of the virus of distemper, and a condition of fever with suppurative catarrh of the respiratory system results, which may mimic certain types of distemper very closely. This was shown by the several discoverers of the organism, and is also recorded by Hardenbergh (1925). But it is now clear that recovery from this infection does not result in immunity to the epizootic disease. It is very probable that a certain number of the recorded double attacks of distemper are accounted for in this way. A foxhound puppy at walk develops fever with catarrhal symptoms which are diagnosed as distemper, but, on return to kennels, this animal does not prove immune to the epizootic disease when it breaks out.

In contrast to the failure of the vaccines made from visible bacteria stand the results of vaccination with products containing dead virus. Puntoni (1923, 1924) propagated the distemper virus by cerebral passage in dogs, and was able to show that the brain of a sick animal might be converted into vaccine by treatment with phenol or formalin. In his earlier experiments he used ' attenuated ' virus after preliminary injection of dead material, but as the attenuated material proved to be unstable, and was, moreover, difficult to prepare, he finally had recourse to killed material only. Three injections of Puntoni's vaccine protected a dog against the injection of living virus. Lebailly (1927) found that the spleens of dogs suffering from distemper might be converted into efficient vaccine by means of formalin treatment, but he did not publish full details of his work. Neither of these observers was able to conduct experiments on an extensive scale, owing to paucity of suitable experimental animals, and the laboratory results were not extended to veterinary practice. The matter was established by the work of Laidlaw and Dunkin (1928[1 & 2]), who, in two different series of experiments relating to ferrets and dogs, showed that vaccines made from virus might be remarkably efficient.

In the first series of experiments it was shown that the spleens from distemper ferrets might be converted into vaccines by killing the virus which was present in them by heat, phenol or formalin. A large dose of killed virus proved to be an efficient antigen, and a single dose of such material was shown to be capable of inducing a resistant state in the recipient, of such a kind that after two weeks' interval some hundred infective doses of living virus might be injected with impunity. A ferret which had received both vaccine and virus proved to have developed a solid immunity, which was durable not only against the strain of virus used to evoke the condition, but also against all other strains which were tested. About 90 per cent. of ferrets could be thus protected, at will, and as the disease is almost uniformly fatal in the unvaccinated animal, the contrast was particularly striking.

In the second series of experiments it was shown that distemper dog tissue might be converted into efficient vaccines by similar treatment, and that dogs which received large doses of suitable dog vaccine became resistant to living virus; further, that dogs which had received both vaccine and virus were solidly immune to all strains of distemper virus which were studied. Control dogs, which had received no vaccine, developed a severe and sometimes fatal disease when injected with a dose of virus equal to the test dose which was innocuous to the vaccinated companion. The immunity induced in this way proved to be durable for months, but it is not yet certain if it lasts for life.

It was found that the vaccine of choice for ferrets was that made from ferret tissues, and that for dogs, from dog tissues. The homologous vaccines were found to be greatly superior to the heterologous. This result is intelligible, without postulating some alteration in the virus with change of host, when it is remembered that the injection of foreign cells and proteins will inevitably prove to be a stimulus to the production of a large number of antibodies at one and the same time. Under the circumstances it is easy to understand that the antigenic response to the noxious agent may easily be overshadowed by the response to the innocuous ones.

The immunity of the recovered dog appears to be due to some cellular alteration, for Laidlaw and Dunkin (1928) failed to demonstrate protective substances in the serum of recovered animals, and convalescent serum has not been shown to be of value in veterinary practice. On the other hand, the serum from dogs which have been intensively hyperimmunized has been shown to contain appreciable amounts of protective substances. Serum which is protective in the case of dogs appears to be without value for ferrets (Laidlaw and Dunkin, 1928).

A prophylactic immunization is being tried at present (Laidlaw and Dunkin, 1928) which consists, first, in the production of a resistant state by the administration of a dose of vaccine, and secondly, the injection of living virus into the resistant animal, in order to convert the resistance into immunity. There is naturally a small risk attached to the injection of living virus, but the risk of death appears to be less than 1 per cent. Over 1,500 animals have been immunized in this way, and although it is too early to form a final judgment, it is clear that many hounds and dogs have been so far protected that they have withstood virulent distemper infection which caused severe and even fatal illness in their unvaccinated companions. It is further clear that some of these animals have proved to be immune for over two years. It still remains to be proved that the hounds and dogs are, like those which have recovered from the natural disease, immune, in the majority of instances, for life.

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CHAPTER XVI. TRACHOMA.

BY J. E. McCARTNEY (METROPOLITAN ASYLUMS BOARD, LONDON), AND M. S. MAYOU (LONDON).

TRACHOMA is a specific disease of the sub-epithelial tissue of the conjunctiva, characterized by infiltration and formation of lymphoid follicles with necrosis. In the later stages of the disease the extensive formation of fibrous tissue of variable amount together with hyaline degeneration takes place. The mere presence of follicles does not constitute trachoma, as any irritant, e.g. atropine, will produce a follicular condition of the conjunctiva. The essential feature of trachoma is the cellular degeneration which is present in sufficient amount to cause the scar tissue on which the diagnosis depends (Mayou, 1905). The clinical diagnosis of trachoma in the early stages is difficult, and only histological examination will prove the presence of the condition. This point is important in considering the results of experimental transmission of the disease.

Bacteriological Investigation.

The causal agent of trachoma is not yet definitely established. The disease can be transmitted from one human being to another, and the incitant, therefore, is presumably a specific micro-organism or virus. Some observers (Royer, 1926), however, regard the condition to be associated with a defective diet, while others (Williams, 1912–13, 1913) believe that it is a chronic inflammatory condition produced by ordinary pathogenic bacteria. The great handicap to experimental work is that no ordinary laboratory animal shows typical trachoma follicles when inoculated with human material. Hess and Römer (1906) produced a condition resembling trachoma in two baboons, while Morax (1911) observed in a chimpanzee inoculated with human material, a mild trachomatous condition that disappeared within two to three months. Further experiments by other workers with anthropoid apes indicated that these animals are either resistant, or else show the disease in a very mild form, which heals rapidly. Nicolle, Cuénod and Blaizot (1911[1 & 2], 1913) claim positive transmission experiments in chimpanzees and in *Macacus* monkeys, the condition sometimes resulting in keratitis, but never pannus. These observers also claim that the rabbit is susceptible to trachomatous material, but this statement is open to much criticism, as rabbits in Tunis are liable to a spontaneous granular conjunctivitis. One of the present writers has attempted to infect young *Macacus rhesus* monkeys with fresh material from active trachoma cases in this country, with completely negative results. The available evidence, therefore, would indicate that none of the ordinary laboratory animals are susceptible, but that in a proportion of cases, inoculation of the higher apes with human trachomatous material may reproduce the disease in a mild degree.

Numerous micro-organisms have been isolated from trachoma cases at various times, and each thought to have a definite causal relationship to the disease, but up to the present no specific bacterium has been proved definitely to be the incitant. The Koch-Weeks bacillus, cocci of various kinds, moulds and yeasts, have each in turn been under consideration, but further investigation has failed to establish any ætiological relationship to the disease. Noguchi (1927, 1928), however, has isolated an organism from human trachoma cases which he termed *Bacterium granulosis*, and for which he claimed a causal relationship to the disease, and this work will be referred to later.

Histological examination of human trachoma material has failed to disclose any recognizable micro-organisms, but certain characteristic intracellular inclusions have been described and have been regarded as identical with or related to the causal agent. Halberstädter and Prowazek (1907) described intracellular bodies in the conjunctival epithelial cells in trachoma cases from Java. They state that in the earliest stages of the disease the organism itself cannot be seen, but that it forms a sheath or mantle which is capable of being stained by Giemsa's method and recognized microscopically. The cellular inclusions appear as small blue coccus-like bodies. Later, the organism is seen in the centre as a minute red granule which increases in size as the disease progresses. The blue mantle gradually disappears and the bodies are seen as a mass of red coccal or cocco-bacillary granules. These bodies are found only in the early stage of the disease, and on account of the sheath or mantle were named *Chlamydozoa* by Prowazek, who regarded them as being intermediate between protozoa and bacteria.

Similar bodies were described by Greeff, Frosch, and Clausen, in Germany (1907, 1908), in trachomatous cases, but apparently identical inclusions have been noted in non-trachomatous conjunctivitis, and even in the urethral cells in chronic urethritis. Moreover, they have not been found in the experimental disease in apes. Park and Williams (1925) consider the inclusion bodies to be degenerated micro-organisms, such as the Koch-Weeks bacillus, or gonococcus, while Noguchi and Cohen (1913) state that other bacteria may also present a similar appearance. It is also possible that some of these bodies may be the debris of cellular degeneration.

It appears, therefore, that inclusion bodies are not peculiar to trachoma, and have no definite relationship to the disease. Their presence does not necessarily indicate a trachomatous condition, neither does their absence exclude it.

The most recent work on the ætiology of trachoma has been done by Noguchi (1927, 1928), and in view of the claims he has made (but which have not yet been confirmed) deserves attention.

Noguchi has isolated from cases of American-Indian trachoma a small Gram-negative motile bacillus, to which he gave the name of *Bacterium granulosis*, and which, when injected under the tarsal conjunctiva of *Macacus* monkeys and anthropoid apes, produces in some cases a granular conjunctivitis which is similar to mild trachoma in the human being. The infection has been transmitted from monkey to monkey up to the fourth passage, and the bacillus can be recovered with difficulty in the primary inoculation and first two passages. Histologically, the lesion resembles American-Indian trachoma, and after prolonged search small bacilli bearing some resemblance to *B. granulosis* can be seen in the tissues. *Bacterium granulosis* is a small Gram-negative non-sporing bacillus measuring 0·8 to 1·2μ long and 0·25 to 0·3μ broad. It is motile under certain conditions and possesses a single terminal flagellum. The organism does not grow on ordinary media, but requires the addition of blood. It thrives best under aerobic conditions at temperatures between 15 to 30° C., in leptospira medium or on carbohydrate blood-agar.

Noguchi considers that *Bacterium granulosis* is the inciting micro-organism of human trachoma and the experimental granular conjunctivitis of monkeys.

After the above results became known, one of the writers examined material from progressive typical trachoma cases, using as far as possible the technique and media employed by Noguchi. After the material was expressed from the follicle it was inoculated immediately into the various media, and the cultures were examined carefully over a period of 14 days' incubation. Up to the present, neither *B. granulosis* nor any organism resembling it has been found.

Cultures of *B. granulosis* in leptospira medium were obtained from the Rockefeller Institute. Morphologically and culturally the organisms present corresponded exactly with Noguchi's description. The actual cultures as received were inoculated under the tarsal conjunctiva in two rhesus monkeys immediately on being received. No reaction was produced, and the conjunctiva in both animals was still normal five months after inoculation.

Discussion.

Noguchi's work requires confirmation not only with trachoma as seen amongst American Indians but also with the disease in the Old World. In his monograph there are several points which are open to criticism. The mere presence of follicles is not diagnostic of trachoma, as any irritant, whether chemical such as atropine, mechanical such as dust, or organismal, may produce a follicular conjunctivitis. The follicles thus produced are aggregations of lymphoid tissue without necrosis. The injection of cultures of an organism of low virulence *beneath* the conjunctiva may produce a reaction resulting in follicles, and this is hardly comparable with the natural disease in man. Noguchi states that in his positive animal experiments the uninoculated eye usually became affected shortly after the inoculated conjunctiva showed a reaction. Clinically a patient may have unilateral trachoma for a period of years without the other side becoming affected. With the human disease, cases of trachoma follow outbreaks of conjunctivitis, and from clinical observations it would seem that trachoma does not supervene on a normal conjunctiva, but that some form of inflammatory reaction is necessary before the condition appears.

The results of animal experiment have not altogether been satisfactory because no criterion has been laid down as to what definitely constitutes trachoma. Clinically the diagnosis of early trachoma by visual examination is difficult in view of the similarity between it and follicular conjunctivitis. In animals this difficulty is not lessened. The question of spontaneous follicular conjunctivitis in monkeys has not been considered, and it does not seem unreasonable to assume that they can suffer from follicular conjunctivitis (as apart from trachoma) due to the same causes as in human beings. The rabbits in Tunis are affected with a spontaneous conjunctivitis, and this fact renders Nicolle's work with these animals invalid.

Histological examination is necessary for the absolute diagnosis of trachoma, and unless this is done fully and from the cytological standpoint, the results obtained cannot be of value. Noguchi's illustrations do not show anything characteristic of trachoma, and precise information regarding the details of cells and fibrous tissue formation is not forthcoming.

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CHAPTER XVII. MOLLUSCUM CONTAGIOSUM.

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MOLLUSCUM CONTAGIOSUM is an infectious disease of the human skin caused by a filterable virus and characterized by the formation of multiple, cutaneous nodules of a pearl-grey or pinkish tint. The outer surface of the nodules is smooth and rounded or flattened, with a tiny central depression from which can be extruded a white caseous substance. The lesions are semi-solid and somewhat elastic to the touch. They have been compared, not inaptly, by Hutchinson (1878) to small pearl buttons. The nodules vary in size from a pin's head to a large pea. Occasionally a pedunculated mass about the size of a hazel-nut is seen, or, more rarely, a reddish growth resembling a small tomato, the so-called molluscum giganteum. The face, arms and genital region are the sites of predilection, though the upper part of the thighs and trunk are not uncommonly attacked. The tongue and mucous membrane of the mouth and lips are on rare occasions the site of the disease ; the palms of the hands and the soles of the feet are never affected. The lesions may be few and localized or numerous and generally distributed. Occasionally about the groins and thighs the nodules coalesce to form irregular patches or sheets, while in rare instances exuberant conglomerate masses simulating epitheliomata are formed.

The infectious nature of the disease was first demonstrated by Paterson (1841), a finding confirmed by Retzius (1871), Vidal (1878), Stelwagon (1895) and others. The disease, although somewhat rare, is more commonly seen in children than in adults, epidemics occurring as a result of close association in schools or swimming baths. In adults several epidemics have been traced to the use of Turkish baths. Paton (1909) has recorded infection as the result of surgical operations. Occasionally infants at the breast have infected their mothers. Ehrmann (1892) suggests that the virus is transmitted by *Pediculus pubis* L. The incubation period varies from 5 weeks to 6 months. The nodules as a rule grow very slowly, requiring from 4 to 6 months to attain a diameter of 1 mm. In infected individuals the lesions may be disseminated by rubbing or scratching. When the disease occurs on the edge of the eyelids there is often an irritating conjunctivitis. The nodules persist for many months but at length tend to undergo spontaneous involution ; they may, however, become infected with bacteria, suppurate and eventually disappear with the formation of scar tissue.

The filterability of the agent of molluscum contagiosum was first demonstrated by Juliusberg (1905), who found that the virus passed through a Chamberland filter, the incubation period being 50 days; these results were confirmed by Serra (1907). More recently Wile and Kingery (1919) have found that the virus also passes Berkefeld filters, the incubation period on inoculation of the filtrate being from 14 to 25 days. Unpublished experiments by the writer showed that the virus passes Berkefeld V and Chamberland L_1 filters, the incubation period on inoculation after filtration being five weeks.

The virus is pathogenic only for man; attempts to infect laboratory animals, monkeys and anthropoid apes have all failed. Neither fowls nor pigeons can be infected, nor is there any evidence to show that human molluscum contagiosum is in any way related to the disease of birds known as avian molluscum, fowl-pox or epithelioma contagiosum. As a result of the failure to transmit the disease to lower animals very little is known in regard to the properties of the virus. It remains active, however, after a month in 50 per cent. glycerin.

Molluscum contagiosum was first described clinically by Bateman (1817), who believed that the material which could be expressed from the nodules was a secretion, which, however, represented the medium of contagion. The histological changes were originally investigated by Engel (1844), who regarded the molluscum tumour as an enlarged sebaceous gland. This view was also adopted by Virchow (1865). Von Bärensprung (1848), Boeck (1875), Campana (1886), Unna (1896) and others maintained the origin of the nodules from the proliferated Malpighian layer of the epidermis. Wile and Kingery (1919) believe that the lesions of molluscum contagiosum are limited to pilo-sebaceous epithelium, a finding which is difficult to reconcile with the occasional development of the disease on the tongue and buccal mucosa. Henderson (1841) and Paterson (1841) were the first to describe rather large oval hyaline structures in the superficial layers of the epithelial nodules. These well-defined structures, which have since been called ‘ molluscum bodies ’, were originally regarded by many observers as of a protozoal nature.

The earliest lesion is a proliferation of the cells of the rete mucosum in one or more areas, which results in downgrowth of the epidermis into the corium in the form of blunt lobulated projections. There is thus formed a rounded somewhat tumour-like mass by which the surrounding uninvolved epidermis is elevated. The nodule is surrounded, except at the surface, by a layer of fibrous tissue in which there is little or no inflammatory reaction. As the growth progresses downwards the papillæ become flattened and remain merely as fine connective tissue partitions separating the wedge-shaped masses of epidermal cells. In the fully developed nodule a cavity appears near the surface of the centre of the growth and gradually extends downwards into each of the lobules. This cavity is filled with the hyalinized ‘ molluscum bodies ’. The development of these molluscum bodies has recently been reinvestigated by Goodpasture and King (1927), whose description corresponds very closely with the account of the development of the virus inclusions in fowl-pox given by Ludford and Findlay (1926), except that in human molluscum contagiosum the virus vacuoles do not acquire a lipoid coat. In the germinal layer of the involved epidermis the only abnormality is an active mitosis and a considerable swelling of the cells. The first significant change occurs just peripheral to the germinal layer. Both cells and nuclei enlarge, as is shown by Sanfelice (1918). The nucleoli become more prominent, and nucleolar material is extruded into the surrounding cytoplasm, where it eventually disappears. In the cytoplasm there appear numerous small vacuoles to the periphery of which are attached small granules. The cell gradually enlarges as a result of the increase in number and size of the vacuoles, while the nucleus is pushed to one side of the cell and becomes flattened and crescentic. The basophilic cytoplasm in which the vacuoles appear condenses in the form of an irregular network between the vacuoles, marking the cell off into compartments. At the same time the vacuoles are filled with minute fairly discrete bodies which represent the minute bodies originally described by Lipschütz (1907).

The finely granular intracellular vacuoles increase in size until they fill the greatly enlarged cell except for the narrow cytoplasmic network between them, and in this fully developed form the entire mass of granules and basophilic cytoplasm appears to separate from a peripheral cellular membrane, and fuses to form an oval hyaline body within a framework composed of its own membrane and the remnant of surrounding keratinized cells. The main factor in the hyalinization of the intracellular mass appears to be desiccation ; it is not formed by keratinization as suggested by Lipschütz. Neither extruded nucleoli nor mitochondria play any part in the formation of the virus vacuoles. The minute granular bodies which appear in the intracellular vacuoles were believed by Lipschütz (1911) to be the actual parasites of molluscum contagiosum and were named by him *Strongyloplasma hominis*. Von Prowazek (1911) also regarded these minute bodies as parasites. In fresh preparations from the epithelial nodules Lipschütz found innumerable non-motile spherical bodies, uniform in size and measuring $0 \cdot 25\mu$ in diameter. These elementary bodies bore no relation to the nucleolar extrusions which Macallum (1892) and Kuznitsky (1895) described within the affected epidermal cells.

Goodpasture and King (1927) find that in smear preparations from the nodules the minute bodies are not demonstrated by ordinary bacteriological stains, but, after fixing in absolute alcohol for one minute, washing in water and mordanting in 1 per cent. potassium permanganate solution, they stain with carbolaniline fuchsine. They appear as deep pink, round or slightly elliptical bodies, often paired as if dividing.

Attempts to cultivate these minute bodies on various liquid and solid media have failed, though they still remain stainable after several weeks when suspended in physiological saline solution at room temperature. The evidence at present available is insufficient to determine whether or not these minute bodies actually constitute the ætiologic agent of molluscum contagiosum. Nothing is yet known as to the development or duration of immunity in this disease, nor has the action on the virus of physical or chemical agents been investigated.

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CHAPTER XVIII. WARTS.

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Definition.

The term wart is somewhat loosely applied to any hypertrophic overgrowth of the Malpighian layer of the skin. Both in man and the lower animals such localized overgrowths may be caused by a variety of agents. Prolonged exposure to X-rays, sunlight, tar, arsenic, or shale-oil may produce in man or in certain animals, such as the mouse or rabbit, warty growths of a precancerous nature. Xeroderma pigmentosum is also characterized by keratotic changes of a precancerous nature, while warts are associated with the condition known as acanthosis nigricans. In rats warts are not uncommon on the ears and nose as the result of an acarus infection, and in rabbits infection with *Spirochæta cuniculi* may cause warty growths. In South America mycotic warts are by no means rare (Chavarria and Shipley, 1927). Localized hypertrophies of the skin arise also from unknown causes as in a cassowary, the head and neck of which were covered with horny nodules. In mice warts are not uncommon, though attempts to determine their ætiology have failed.

In the present article only those warts are described in which there is evidence to show that a filterable virus is of ætiological significance. Such warts occur in man, dog and cow. Certain wart-like growths in the frog and horse may also be due to filterable viruses.

Human Warts.

While many dermatologists of the nineteenth century were wont to scoff at the idea that warts are infectious, folk medicine has long believed in their extreme contagiousness. Their habit of occasionally appearing or disappearing with remarkable suddenness probably gave rise to the idea that they could be 'wished' or alternatively 'charmed away' (Wright, 1928).

Clinically it is usual to distinguish several varieties of warts.

The typical plane or juvenile wart—*verruca plana seu juvenilis*—occurs chiefly on the face, neck or dorsum of the hands, often in very large numbers. Although usually found in young people the warts may occur in adults. They are small, smooth elevations of somewhat irregular outline, varying from 1 to 3 mm. in diameter and 0·5 mm. in height. They are diffuse or localized, and, though generally the colour of the normal skin, they may on occasion be white or yellowish brown. When viewed with a hand-lens the surface is sometimes seen to have a 'raspberry' appearance.

The common wart—*verruca vulgaris*—usually occurs on the dorsal surface of the hands and fingers of children and more rarely of adults. Not infrequently plane warts are present on the face, common warts on the hands. Common warts are also found at the side of the nails, involving the nail-bed, on the palms, forearms or other areas. They vary in number and may be isolated or grouped. At times a single mother-wart is followed by the development of many others. They vary from 1 to 10 mm. in diameter ; the outline is round, oval or elongated. In their early stages the warts are smooth, horny elevations, the colour of normal skin ; in time the surface becomes roughened with minute horny projections, the colour yellowish-brown or black.

Plantar warts—*verruca plantaris*—were first described by Dubreuilh (1895). They occur on the soles of the feet chiefly under the ball of the great toe, under the pad of the toe itself, or on the heel, areas which are subjected to pressure. They are especially common in athletes who wear thin-soled shoes for walking. An analogous form is found on the finger-tips of seamstresses. These plantar warts often resemble a corn or callosity and from pressure on the nerve-endings may be exquisitely painful. Their development has been described in the centre of corns following the operations of a chiropodist.

Digitate warts—*verruca digitalis*—are found on the scalp, neck and face. As the name implies these warts are made up of soft finger-like processes with a horny cap.

Filiform warts—*verruca filiformis*—are similar to the digitate form though more circumscribed. One or more delicate thread-like elevations are present either on the eyelid or face.

Condylomata acuminata, moist, or fig warts, have for long been known as venereal or gonorrhœal warts. They are found on the genital regions ; in the male on the glans penis around the frenum and on the prepuce ; in women about the clitoris and labia, on the perineum or about the anus. Growths having an exactly similar appearance may on occasion be found in the axillæ, around the umbilicus or between the toes, and even on the tongue and buccal mucosa.

Condylomata acuminata vary greatly in size, occasionally becoming as large as an egg. They form luxuriant cauliflower-like growths of a pink or brownish colour, sessile or pedunculated. As a result of the moisture of the regions in which they grow their surface tends to become covered with secretions having an offensive odour. The secretions dry, forming a brownish crust upon the growths. Occasionally the lesions, flattened by pressure, simulate condylomata lata. In pregnant women these warts sometimes grow rapidly, disappearing suddenly after parturition.

Senile or seborrhæic warts—*verruca senilis*—are found on the back, arms, and less commonly on the face and chest of old people. The lesions are flattened and only slightly elevated. They are covered with a friable crust of a brown or black colour. On removing the crust the surface is found to be red and slightly papillomatous. These warts occasionally become malignant.

Cases of generalized *verrucosis of the scalp* have been described by Hoffmann (1926) and Glaubersohn (1928); the condition is probably allied to Lewandowsky's epidermo-dysplasia verruciformis (Lewandowsky and Lutz, 1922).

Histology. The essential change in all types of wart is, as was shown by Unna (1896), an active proliferation of the Malpighian layer of the skin resulting in the formation of a surface projection, and an elongation and broadening of the interpapillary processes. In addition there is usually some thickening of the stratum corneum as well as parakeratosis. If by pressure on the skin the newly formed tissue is prevented from causing a surface projection it projects inwards and depresses the corium. As Roxburgh (1928) points out, varying degrees and combinations of these secondary features account for the different varieties of warts. In addition some cellular infiltration may be present in the corium, though this is not a prominent feature, except in condyloma acuminatum.

In the plane wart the chief change is in the rete mucosum, there being only slight hyperkeratosis. A similar appearance is found in the early stage of the common wart, but in the later stages the interpapillary projections of the prickle-cell layer are drawn out and irregular. The papillæ are correspondingly elongated and each is distinguishable on the surface with its hyperkeratotic cap. In plantar warts owing to pressure the interpapillary processes are forced inwards so as to invaginate the corium, which becomes condensed. The opening is as a rule narrower than the bottom of the pocket containing the wart.

In filiform, digitate and acuminate warts the structure is very similar. The stratum corneum is but little thickened and in some instances is thinned. The interpapillary projections are greatly lengthened, the cells showing very active mitosis. The papillæ are much enlarged and often branched, with dilated blood-vessels and lymphatics. In the corium there is a considerable infiltration of polymorphonuclear leucocytes, plasma and mast cells.

The histology of senile warts was described by Unna (1896), Hartzell (1903), and others. Sutton (1928) describes three types: (1) a keratoid variety characterized by great corneous hypertrophy, some parakeratosis, a moderate degree of acanthosis, slight proliferative changes in the germinal layer and more or less flattening of the papillary bodies; (2) a nævoid type practically identical with Unna's 'nævus seborrhœicus'; (3) a verrucose form associated with considerable hyperkeratosis, very pronounced acanthosis, active proliferation throughout the rete and enormous papillary hypertrophy. The cutis also shows inflammatory changes of a subacute nature with capillary dilatation and perivascular infiltration.

Ætiology. It has long been popularly believed that if a wart is made to bleed the blood will induce fresh crops of warts. During the nineteenth century certain clinical observations, such as those of Payne (1891), were published in support of the contagiousness of warts. Variot (1893), however, was the first to inoculate warts from a child directly to an adult, a result which was confirmed by Jadassohn (1896), who out of 74 inoculatory tests on the skin of the hands obtained 41 positive results. Similar results have been obtained by Lanz (1899), Juliusberg (1903), and others. Lanz found that the incubation period was 6 weeks ; Juliusberg, 50 days.

The filterability of the agent of warts was first proved by Ciuffo (1907), who used a Berkefeld N candle. These results were confirmed by Serra (1908), who found that the virus passed through a Berkefeld W candle, lesions developing in from 5½ to 6 months. Wile and Kingery (1919), after filtration through a Berkefeld candle, found that sterile filtrates induced lesions in 4 weeks. Kingery (1921) succeeded in producing a second generation of warts by inoculating a filtrate of warts originally produced by inoculation, the incubation period being about 6 months. Experiments by the writer have shown that there is considerable variation in the length of time necessary to produce lesions with a filtrate of warts through a Berkefeld V candle. This variation, extending from 3 to 8 months, appears to be due to variation in individual susceptibility.

Although condylomata acuminata have for long been regarded as due to gonorrhœa, the evidence is now in favour of the view that they are due to the same ultramicroscopic virus as causes the other varieties of warts. Cooper (1845) states that a doctor when removing a large acicular condyloma with his instrument, injured his assistant beneath the thumb-nail. After a short time a wart appeared on the injured place. Rasch (1896 and 1900) has maintained the contagiousness of condylomata acuminata on clinical grounds, such as the simultaneous appearance of common warts on the hands and acicular condylomata on the genitals. Brandes (1925) also noted that of 38 patients with condyloma acuminatum, 26 had verruca vulgaris as well, while more than half had no gonorrhœa. Waelsch (1918) inoculated healthy skin and mucosa with condyloma acuminatum and obtained flat warts on the skin and papillomata on the mucosa. Serra (1924) found that the Chamberland filtrate of condylomata acuminata gave rise to warts on the hand. This result has been confirmed by the writer who found that the virus could pass a Berkefeld V filter.

The ætiology of senile warts still remains obscure. Though certain senile warts may be due to an ultramicroscopic virus, others possibly are of a precancerous nature.

With the exception of the fact that the virus of warts withstands the action of 50 per cent. glycerin, nothing is known as to its reactions to physical or chemical reagents. Crabtree (1928) has found that the tissues of warts possess a power of aerobic and anaerobic glycolysis similar to that observed with tumours and fowl-pox lesions.

Laryngeal Papillomata.

Werner (1894) and Thost (1911) have drawn attention to the frequency with which laryngeal papillomata coincide with warts on the skin of the face and hands. Ullmann (1923) has brought forward evidence in favour of the view that laryngeal papillomata are due to a filterable virus identical with that of warts. By inoculating a laryngeal papilloma on to human skin he obtained on the epidermis of the arm papillomatous growths, but on the scalp and face flat warts. The papilloma from the arm was carried on to the third generation, the period of incubation decreasing from 2 to 4 months to 4 to 5 weeks. Using a bacteria-free filtrate 2 positive results were obtained out of 6 experiments. If these results are confirmed they open up the question whether papillomatosis of other organs, such as the bladder, intestine or ovary may not be due to an ultramicroscopic virus.

Warts in Animals.

Canine warts. Warts in dogs are by no means rare. In old dogs one or more warts may be present on the skin. Attempts by the writer to infect other dogs with these senile warts have failed. In younger dogs, and especially in puppies, a diffuse papillomatosis of the mouth occasionally occurs. Epidemics among foxhound puppies have been described by Penberthy (1898) and others. While constantly present in some kennels, in others the disease never occurs. The warts are found often in large numbers on the lips, tongue, particularly the dorsum, palate, pharynx and buccal mucosa. Occasionally warts may also be present on the skin. The growths are usually discrete and fairly uniform in size and shape, the colour being slightly paler than the buccal mucous membrane. On microscopical examination considerable acanthosis with hyperkeratosis is seen. The papillæ are elongated with dilated blood-vessels. The contagiousness of these warts was demonstrated by M'Fadyean and Hobday (1898), who found that they could be transferred by rubbing the scarified buccal mucosa with the cut surface of a recently excised wart. The incubation period varied from a month to six weeks. The warts after some few weeks disappeared spontaneously. Unpublished observations by the writer have confirmed the findings of M'Fadyean and Hobday. The causal organism is filterable through Berkefeld candles, and the disease may be propagated in series by means of the filtrate. The virus withstands 50 per cent. glycerin for at least two months.

Bovine warts. Warts may occur in cattle as a diffuse papillomatosis of the nostrils, limbs, belly and back, or may be restricted to the udders. They are sessile or pedunculated, fleshy or hard in consistence. Schindelka (1908) found that it was possible to transmit bovine warts by rubbing freshly collected material into the scarified skin of calves. Magalhães (1920) infected healthy steers intravenously with an emulsion of warts derived from a case of general papillomatosis. The emulsion gave rise to a generalized infection after an incubation period of three weeks. Filtrates through a Berkefeld candle also gave positive results. The writer has found that a Berkefeld filtrate of warts from the udder gave a positive reaction after five weeks. The virus withstands the action of 50 per cent. glycerin.

Amphibian warts. Sanfelice (1913) has described a form of warts in *Discoglossus pictus*, a frog from the Province of Catania, Italy. The warts, small whitish nodules which may be discrete or confluent, vary considerably in numbers. They are found chiefly on the back and abdomen. Nothing is known as to the infectivity of the condition.

The Relationship of Human and Animal Warts.

Serra (1924) was unable to infect laboratory animals with human warts. Ullmann (1923) claims to have produced a papilloma in the vagina of a bitch by inoculation with a laryngeal papilloma. The writer has been unable to confirm these results. Dog warts failed to infect man, rabbits, mice or guinea-pigs, as did bovine warts. Human warts were not infective for dogs, rabbits, rats, mice or guinea-pigs.

Immunity.

Spontaneous disappearance of human and of canine warts is by no means uncommon. Certain persons inoculated with human warts fail to react. The writer found that after inoculating himself with three crops of warts he became immune to further inoculation. The serum, however, had no antiviral properties, but after hyperimmunization acquired them. Dogs are also capable of producing an antiviral warts serum, but a serum which acts on human warts has no action on dog warts and vice versa.

Virus Inclusions.

From time to time virus inclusions have been described in warts. Sanfelice (1913) found intranuclear inclusions in the warts of *Discoglossus pictus*. The small and middle-sized bodies took on a reddish stain, the larger ones a deep blue colour by Mann's method. Sangiorgi (1915) described in human warts acidophilic cytoplasmic inclusions which are almost certainly nucleolar extrusions. Lipschütz (1924^1) finds in warts nuclear inclusions which are basophilic. Ullmann (1923), on the other hand, found acidophilic nuclear inclusions like those of herpes in papillomata of the dog's vagina. The presence of these acidophilic bodies and also of cytoplasmic inclusions has been confirmed by Lipschütz (1924^2). Kyrle (1925) regards these inclusions as an oxychromatic change of the nucleoli. Similar nucleolar changes are present in early psoriasis lesions, a finding confirmed by Lipschütz (1926), and also in rabbit warts caused by *Spirochæta cuniculi*.

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CHAPTER XIX. FOWL-POX.

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FOWL-POX is an acute infectious disease of birds caused by a filterable virus and characterized by hyperplastic nodular lesions of the skin and its appendages, diphtheritic membranes in the mouth and throat and oculo-nasal discharges. The lesions may be confined to the epidermis, to the mouth and throat or to the nasal mucosa and conjunctiva.

Historical.

In mediæval times, according to Burnet (1906), fowl-pox was believed to be identical with smallpox and to be transmissible to man. This view was questioned by Spinola (1858), who failed to transmit cow-pox to birds, and was discarded on the discovery by Rivolta (1869) of the characteristic cell inclusions, and as a result of the histological investigations of Bollinger (1873), who likened the disease to a neoplasm and termed it epithelioma contagiosum. The cell inclusions were believed to be of a protozoal nature until Marx and Sticker (1902) proved that the causative agent was a filterable virus. This virus was cultivated by Findlay (1928^2) in association with growing tissue cells.

Relationship to Avian Diphtheria, Roup, Molluscum Contagiosum and Epithelioma.

Fowl-pox was believed to be entirely distinct from avian diphtheria until Carnwath (1908) proved that material from the membranous lesions in the mouth and throat could give rise to typical fowl-pox eruptions on the skin, findings confirmed by Schmid (1909), Sigwart (1910), Uhlenhuth and Manteufel (1910), Betegh (1913), van Heelsbergen (1920) and others. The virus, however, is present in lesser concentration in the lesions of the mouth than in those of the skin, probably because in the former site it is more rapidly destroyed by the overgrowth of saprophytic bacteria. Other agents may, however, produce the clinical picture of avian diphtheria. Various observers claim to have isolated bacteria which on inoculation produce symptoms of avian diphtheria but not of fowl-pox.

It is uncertain whether fowl-pox is identical with roup, by which is generally understood a catarrhal condition of the conjunctiva and of the mucosa of the upper air-passages. Haring and Kofoid (1912), Beach (1920), van Saceghem (1922), Brunett (1924) and Gwatkin (1924) believe the diseases to be distinct. Biró (1913), however, regards 'contagious catarrh' as a manifestation of fowl-pox. According to Ward and Gallagher (1920) roup is readily transmissible by inoculation of material from infected birds, the virus of fowl-pox being occasionally associated with the condition. Doyle and Minett (1927) also conclude that the virus of fowl-pox may be associated with roup, although an attack of roup does not necessarily confer a high degree of immunity against fowl-pox. In one instance virus isolated from the nasal discharge of three chickens gave rise to typical fowl-pox lesions in healthy fowls, but the original chickens were susceptible to fowl-pox one month later. The question of relationship of fowl-pox and roup is complicated by the fact that lack of vitamin A produces in the fowl a condition closely resembling roup. Bushnell and Hinshaw (1924) in America, and Rice (1926) in Ireland, have described epidemics of 'roup' due to lack of vitamin A, while McCarrison (1923) found that pigeons fed on polished rice are more susceptible to the fowl-pox virus.

If 'roup' is not to be merely a clinical term it seems necessary, as Doyle and Minett suggest, to restrict it to the catarrhal inflammation of the conjunctiva and upper air-passages when these are unaccompanied by lesions of fowl-pox in any bird of the flock.

There is no evidence that fowl-pox is related to molluscum contagiosum in man, nor is there any connection with the type of tumour known as an epithelioma, a form of neoplasm rare in birds. The virus of fowl-pox has, however, many analogies with the agent causing Rous sarcoma of fowls, and with that group of organisms which includes the viruses of warts, human molluscum contagiosum, and leukæmia of fowls, all of which are capable of inducing cell proliferation.

Species Affected.

The disease is most commonly seen in fowls and pigeons, but Ward and Gallagher (1920) describe it also in turkeys, geese, ducks and guinea-fowl. In this country the disease is rare in ducks, though in France it is more common. Doyle and Minett (1927) and Findlay (1928[1]) failed to infect ducks with virus derived from fowl and pigeon. Among wild birds the condition has been met with in quails (Gallagher, 1916)—the virus inoculated into fowls producing lesions similar to, though less extensive, than those caused by 'fowl' virus. Ticehurst (1908) has recorded epidemics of avian diphtheria in wood-pigeons. Dean and Marshall (1909) showed that a filterable virus could be obtained from the diphtheritic lesions. True fowl-pox has also been noted in the hawk, pheasant, partridge (Findlay, 1928), and bunting sparrow (Shattock, 1898).

The virus is not pathogenic for man (Pfeiffer, 1889; Juliusberg, 1904), nor for goats and sheep (Bollinger, 1873). Efforts to infect mice, rats and guinea-pigs have all failed. Intradermal inoculation of the virus in rabbits has with the majority of workers produced a non-specific temporary redness, though Levaditi and Nicolau (1923) report papules characterized by perivascular infiltration and epidermal proliferation. Lusena (1926) found that the virus could survive without causing symptoms in the organs and blood of the rabbit for 65 days after inoculation. In pregnant rabbits the blood and tissues of the fœtuses become infected with the virus. van Heelsbergen (1920 and 1922) claims to have infected the rabbit, calf, cow and horse, the lesions in the mouth of the horse closely resembling those of pustular stomatitis. Toyoda (1924) professes to have transmitted fowl-pox virus directly to the rabbit, sheep and guinea-pig, while after passage through the rabbit a child was infected. van Nederveen (1926) also infected the rabbit and calf, and, after passage through the latter, man. Pandit (1927) failed to infect the rabbit except after passage through the calf, but succeeded in infecting monkeys. Findlay (1928) failed to infect rabbits and calves. The frog and tortoise are also resistant. In many of these experiments insufficient attention has been paid to the possibility of contamination of the virus of fowl-pox with that of vaccinia. A critical repetition of the experiments of Toyoda, van Nederveen and Pandit with numerous strains of fowl-pox is indicated.

Epidemiology.

Epidemics of fowl-pox have been recorded from all European countries, from Asia and America. Galli-Valerio (1925) in Switzerland, however, only encountered one case in 27 years. The disease may suddenly appear in epidemic form in flocks where for some years only an odd victim has occurred, while the mortality varies greatly in different outbreaks. Contact between diseased and healthy birds is essential for infection, since the usual method of propagation is probably through wound infection. Such small wounds frequently result from the pecking and fighting inseparable from a flock, while abrasions of the mouth are common as the result of eating grit. Burnet (1906) found that virus placed in the drinking water for one month failed to infect a pigeon except when sharp grit was fed at the same time. Occasional success in infecting pigeons was obtained by feeding them on grain soaked with virus. Direct inoculation of the virus into the embryo-containing egg neither infects nor immunizes the developing chick. According to Schuberg and Kuhn (1912) successful transmission of fowl-pox by *Stomoxys calcitrans* has been obtained. Kligler, Muckenfuss and Rivers (1928 and 1929) noted passive transference of the virus by *Culex* and *Aëdes* mosquitoes.

Though epidemics may occur at any season of the year the disease is more frequent in spring and autumn.

The Natural and Experimental Disease.

In the naturally occurring disease nodular lesions appear first on the comb, wattles, eyelids, angles of the beak and oral orifices, except in very severe cases when any portion of skin, feathered or unfeathered, may be affected. At first the skin lesions are small, firm, greyish or pink nodules, which rapidly increase in size, from about 1 mm. to about 0·5 cm. in diameter, become rough, brown or yellow in colour, and horny in consistence. At first the nodules may be many or few, for all do not erupt at the same time. If the bird survives, the nodules in from 2 to 3 weeks dry up and some weeks later fall off as crusts without leaving a scar, except in severe cases. When the conjunctiva is affected it appears swollen, while a serous discharge pours from the conjunctival sac. Keratitis may occur. Greyish white patches of false membrane may appear on the mucous membrane of the mouth and pharynx and on the sides of the tongue constituting the so-called avian diphtheria. In geese, lesions are said to occur on the webs of the feet. The walls of the œsophagus and crop sometimes exhibit similar lesions. In fatal cases there is emaciation, associated with anæmia.

The disease can be transmitted experimentally by :

(i) Applying virus to the scarified comb. Numerous small vesicles first appear followed rapidly by a pustular stage. The disease rarely becomes generalized.

(ii) Applying virus to the skin from which feathers have been plucked. Within 48 hours the feather follicles are swollen and pale yellow.

(iii) Applying virus to the scarified tongue or buccal mucosa.

(iv) Instillation into the conjunctival sac.

(v) Intravenous injection. After an incubation period of from 1 to 2 weeks lesions appear on the unfeathered portions of the skin and on the buccal mucosa. Occasionally no lesions appear: immunity may be acquired or the bird may become emaciated and die.

(vi) Intradermal inoculation into the comb.

(vii) Subcutaneous, intramuscular or intraperitoneal injection. The results are similar though less widespread than those of intravenous injection.

Application of the virus to the unbroken comb, skin or buccal mucosa fails to infect, as does feeding with gelatin capsules containing the virus. Intracerebral inoculation produces, according to Levaditi and Nicolau (1923), a mild form of meningo-encephalitis.

Histological and Cytological Changes.

When the virus is applied to a scarified area, or to one from which the feathers have been plucked, there occur during the first 24 hours intense congestion in, and swelling of, the endothelial cells of the capillaries in the dermis, accompanied by an exudation of round cells and polymorphonuclear leucocytes and a proliferation of the adventitial cells. At the end of 48 hours, in addition to the dermal reaction, active mitosis is noticeable in the epidermal cells of the basal layers, while many of these cells contain the earliest stages of the virus bodies. As a result of the active mitosis irregular hyperplastic masses of ballooned epithelial cells are formed. When, following intravenous inoculation, nodules appear at the sites of election, the dermal reaction is less in evidence. Injection of histamine into any area of skin leads to localization of the virus in that area following intravenous inoculation of the virus (Findlay, 1928[3]).

Attention has been largely directed to the virus bodies, which are restricted to the cytoplasm of the epidermal cells of the fowl-pox lesions. These ' Bollinger bodies ', so called from their early description by

Bollinger (1873), were probably first noted by Rivolta (1869). They are fully discussed in Chapter II. Woodruff and Goodpasture (1929) have recently shown that a single Bollinger body washed in saline and inoculated into the skin of a hen will produce typical fowl-pox.

The microscopical changes in the lesions of the buccal mucus are similar to those in the skin though bacterial infection of the infected epithelium occurs at an earlier stage. There are no characteristic changes in the internal organs.

Distribution of the Virus in the Body.

During the course of the disease virus is present in the blood in variable amounts. There is no evidence that the virus multiplies in the blood-stream, and, since, in the naturally occurring disease, virus is not present in the blood until after the development of epidermal lesions, it seems probable that it passes into the blood from these foci. Virus is also found in the liver, spleen, ovary, lungs, brain and kidneys, though virus bodies do not occur in these organs. It is not present in the urine or fæces. According to Sanfelice (1914) virus is present in the liver and kidney within 24 hours of inoculation on the scarified skin : it remains in the body for as long as 71 days.

The Virus.

No accurate account can be given of the size of the virus. Borrel (1904) was the first to describe minute granules within the virus inclusions. In filtered emulsions of the epithelial nodules similar small spherical or biscuit-shaped non-motile bodies, $0 \cdot 25\mu$ in diameter, have been described by Lipschütz (1921) and others, who believe that these bodies represent the virus. Examination by the most refined optical methods of infective blood, both in the fresh condition and in stained preparations, fails to reveal any granules which are absent from normal blood. Thus the granules described by Borrel may equally well represent aggregates of colloidal protein molecules by which the virus is adsorbed.

Lewis and Andervont (1927) find that the virus is adsorbed by kieselguhr, kaolin and wood charcoal. It is inactivated by 75 per cent. of indian ink, 50 per cent. of baked kieselguhr and 20 per cent. of kaolin, but not by carmine, as are vaccinia and the Rous sarcoma virus.

Dried virus retains its activity at room temperature for from 6 months to 2 years. Burnet (1906) found that it was not destroyed after 2 months by diffuse daylight. It was killed by 60° C. in 8 minutes and by 12° C. in 5 weeks. At 56° C. it was destroyed in 30 minutes. When suspended in water at 37° C. it was inactive after 8 days. It is destroyed by 20 minutes' exposure to ultraviolet light, being more resistant than *B. prodigiosus*. It remains active after from 6 months to 1 year in 50 per cent. glycerin. Friedberger (1918) has studied the lethal effect of all the commoner disinfectants. Manteufel (1910) finds that bile decreases the virulence of the virus. Crabtree (1928) has recently found that skin infected with the virus acquires a power of aerobic and anaerobic glycolysis similar to that which Warburg (1926) believes to be characteristic of neoplastic tissues.

Filterability.

Marx and Sticker (1902) found that the virus was capable of passing through a Berkefeld filter but not a Chamberland F. These results have been confirmed by Juliusberg (1904) and Burnet (1906). Ledingham (1926) and Findlay (1928) showed that the virus passed Berkefeld V candles with a reduction in potency, though it was retained by Chamberland L2 candles. It is difficult to compare the results of different workers owing to the lack of standardization of such factors as the pressure, time of filtration and amount of virus present in the suspension before filtration.

Cultivation.

Bordet and Fally (1910) cultivated a small bacillus from a case of avian diphtheria. They produced diphtheria in birds with this organism but never fowl-pox. It seems probable that the disease from which their organism was isolated was some form of roup, as others have failed to isolate a similar organism from true fowl-pox. Findlay (1928[2]) using tissue cultures of chick embryo brain and skin as a substrate obtained multiplication of the virus *in vitro*.

Immunity and Serology.

It has long been recognized that birds which have recovered from a severe attack of fowl-pox are thereby rendered immune. By the fourth day there is slight immunity which is complete by the twentieth day. This immunity persists for a considerable time, being but little reduced 150 days after the onset of the disease. It is possible that certain fowls and pigeons are naturally immune to infection with fowl-pox. Immunity occurs equally when the disease has affected the skin, mucous membrane, or conjunctival sac. It appears to be largely of a cellular character, since serum of immune birds has but little if any anti-viral action. As Dalling, Allen and Mason (1925) and Findlay (1928[2]) have shown, however, by hyperimmunization it is possible to produce sera with anti-viral properties. There is no evidence of an agglutinating or precipitating action by immune sera. Experiments on complement fixation have been reported by Haring and Kofoid (1912), who obtained positive results with antigens of infected fowl-liver or extracts of the lesions. Saito (1926) failed to confirm these results.

Dalling, Mason and Gordon (1929) have used immune serum prophylactically and therapeutically with success. Many attempts to produce immunity by the use of killed virus have been made, notably by Manteufel (1910), Hadley and Beach (1913) and Boerner and Stubbs (1921). Ward and Gallagher (1920) and Gwatkin (1924) failed to obtain any protection with these vaccines. De Blieck and van Heelsbergen (1922) claim more successful results with an attenuated virus, which occasionally, however, produces generalization of the disease. Broerman and Edgington (1929) recommend the use of a phenol-glycerinized vaccine. The virus is still active, however, there being little or no evidence that killed virus has any immunizing power.

The Potency of Fowl-pox Virus.

While strains of fowl-pox derived from the pigeon are virulent both for the pigeon and the hen, strains of virus obtained from epidemics in the hen are, as a rule, either avirulent or but slightly virulent for the pigeon. Carnwath (1908) and Basset (1926) failed to infect pigeons with virus derived from the hen, as did Findlay (1928) using two hen strains. Galli-Valerio (1925) on the other hand found that infection could be easily transmitted from fowl to pigeon. Saito (1926), Doyle and Minett (1927) with considerable difficulty transferred hen virus to the pigeon. The relationships of hen and pigeon strains of fowl-pox virus were re-investigated by Findlay (1928). Two strains from hens and one strain derived from the pigeon were studied. Passage of the pigeon strain in the fowl only slightly and temporarily attenuated its virulence for the pigeon, though Marx and Sticker (1902) report that after a single passage through the hen the virulence for the pigeon is either greatly reduced or completely destroyed. In the fowl any one of the three strains completely immunized against the others, but in the pigeon previous treatment with strains pathogenic for the hen produced only partial immunity against the strain pathogenic for pigeon and hen. An anti-viral serum prepared against one of the hen strains destroyed the pathogenicity of both the viruses which were pathogenic for the fowl only, but when acting on the virus pathogenic both for hen and pigeon, destroyed only its pathogenicity for the fowl. An anti-viral serum prepared against the pigeon strain destroyed the pathogenicity of all three strains. The relationship of strains of fowl-pox virus derived from different sources requires further investigation.

The Relationship of Fowl-pox to Vaccinia and other Pox Viruses.

Although earlier observers, relying possibly on the popular superstitions of the middle ages had assumed that fowl-pox was identical with small-pox in man, the discovery of the characteristic cell inclusions of fowl-pox seemed to render this view untenable. Recently, however, certain workers have returned to the view that fowl-pox is a form of vaccinia virus slightly modified by passage through birds. The evidence bearing upon this point is as follows :

(i). *Cytological.* Ludford (1928) has shown that in young chickens vaccinia produces cytological changes which cannot be differentiated from those caused by fowl-pox. But vaccinia passaged in the chick does not lose its power of infecting the rabbit or rat cornea.

(ii). *Immunological.* van Heelsbergen (1920), Toyoda (1924), van Nederveen (1926) and Pandit (1927) claim to have transmitted fowl-pox to rabbits, calves, monkeys and man with subsequent immunity to vaccinia. On the other hand, Levaditi and Nicolau (1922), found that though the virus of vaccinia proliferated in the lesions of fowl-pox there was no cross immunity. The absence of cross immunity has been confirmed by a number of subsequent observers. Findlay (1928) found that an anti-vaccinia serum from the hen or rabbit had no anti-viral action on the virus of fowl-pox, nor had an anti-fowl-pox serum from the hen any action on vaccinia virus.

(iii). *Filterability.* While fowl-pox virus passes a Berkefeld filter, vaccinia virus, even after repeated passage in the chick, is retained.

(iv). *Reaction to physical agents.* Lewis and Andervont (1927) have found that vaccinia virus is more readily adsorbed than fowl-pox.

The weight of evidence is, therefore, in favour of the view that vaccinia is distinct from fowl-pox, but, since there appear to be differences between various strains of true fowl-pox virus, it is always possible that certain strains of fowl-pox may have some affinity with vaccinia. Apart from accidental confusion of the viruses, the results obtained by Toyoda, Pandit and van Nederveen may be due to an infection of the original hens from which these observers obtained their material with the virus, not of true fowl-pox, but of vaccinia. Investigation of a larger number of strains of fowl-pox virus obtained from different sources must obviously be carried out before the question of the relationship of fowl-pox to other pox viruses can be definitely settled.

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CHAPTER XX. THE FILTERABLE TUMOURS OF FOWLS.

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The existence of tumours which can be propagated by means of cell-free filtrates was discovered by Peyton Rous in 1911. The first of such tumours to be described was a simple spindle cell sarcoma, which occurred spontaneously in a fowl of the variety known as Plymouth Rock. Propagation by means of cell grafts was in the earliest generations restricted to blood relations of the fowl in which the spontaneous tumour appeared. Later, this fastidiousness was lost. To-day, this tumour—known as Fowl Tumour No. 1 of the Rockefeller Institute series—grows readily in any variety of domestic fowl. The tumour normally grows very rapidly—a graft of 0·05 c.cm. growing in three weeks to a size of 40 to 60 c.cm.—forming a soft hæmorrhagic mass with a central cavity containing blood and mucus-like fluid into which fungating masses of tumour grow. The normal parts, particularly muscular tissue, become invaded and replaced by sarcoma cells. Sometimes the growth of the tumour is accompanied by widespread necrosis of normal tissues which are replaced *en bloc* by a solid mass of new growth. When the tumour grows slowly, as, for example, in resistant fowls, or during the occasional periods of depressed proliferative power, or following the injection of filtrates damaged by heat or antiseptics, the tumour formed is firm in consistence, white to yellow in colour and surrounded by a capsule. Metastasis formation is common, the sites in order of frequency being the lungs, heart, liver and spleen. The tumour has retained its original macroscopic and microscopic characters.

It was shown by Peyton Rous that cell-free filtrates of this tumour are able, when injected into the breast muscles of sensitive chickens, to cause the formation of a new tumour identical in all respects with the parent tumour. The filtrate is made by disintegrating tumour tissue with sand, diluting with saline, which, after spinning, is filtered through a bacteria-proof candle. Berkefeld N or Mandler candles are usually employed ; the Chamberland L2 candle is useful for small quantities of fluid. Filtrates through Chamberland L5 candles are inactive. Active filtrates contain no cells and the tumours caused by their action on normal fowl cells are therefore strictly new tumours. In this respect they differ from the propagable mammalian tumours, which are transmitted only by means of living cells and give rise to daughter-tumours which are formed exclusively by division of the implanted tumour cells. If it could be shown that the filterable fowl tumour No. 1 is a true tumour and not of the nature of a granuloma, the discovery of its filterability is of cardinal importance in destroying the almost universal opinion that no extrinsic cause—other than secondary causes such as irritation—plays a part in the evolution of new growths.

During a prolonged study of this question, Rous, Murphy and their collaborators at the Rockefeller Institute, investigated more than forty tumours of fowls. Five of these were found to be propagable by means of filtrates and glycerinated tumour tissue, and all but one by means of dried tumour.

Fowl tumour No. 7 was discovered as a hard, slow-growing, apparently benign, nodule on the breast bone of an otherwise healthy Plymouth Rock hen. With continued transplantation the tumour, an osteochondrosarcoma in structure, increased slightly in infiltrative power but otherwise remained true to type. Its rate of growth was slower than that of tumour No. 1. Metastasis formation was rare but did occur. Injection of cell-free filtrates was followed by the development of tumours of the same structural and biological character as the original growth. Active dried tissue was difficult to prepare. The causative agent could withstand glycerination for at least thirteen days.

Fowl tumour No. 18, in structure a sarcoma rifted with blood sinuses, occurred in a mongrel brown Leghorn hen and was probably primary in the gizzard. With repeated transfer by means of cell grafts the rate of growth of the tumour increased. Metastasis formation occurred in the skeletal muscles usually near a joint. Cell-free filtrates were active in giving rise to new tumours of characteristic structure and properties, but attempts to obtain the virus in active form in dry or glycerinated tissue, failed.

Tumour No. 38 was a filterable tumour practically indistinguishable from No. 18, except that dried and glycerinated tissue was active.

Tumour No. 43 resembled tumour No. 1 in its gross appearance, microscopic structure and easy filterability.

Rous and his colleagues proved that these filterable tumours are true neoplasms by showing that they possess all the properties which are regarded as characteristic of true tumours. This is shown in the histological types of structure and in the infiltrative and expansive method of growth; in growth being independent of the needs of the host; in the absence of inflammation around the nodules; in the mode and routes of metastasis formation; in the strictly localized character of the disease, both at primary and secondary sites; in the mode of retrogression in resistant hosts; in each strain possessing characteristic sites of election of metastasis formation; in transplantability and in having a latent period and rate of growth more or less distinctive of the individual strain of tumour; in being strictly specific for the species and in exhibiting variable malignancy even in one and the same tumour strain. In all these properties and in many others no difference was found between these filterable tumours and the non-filterable tumours of mammals.

Filterable fowl tumours have been found by many other observers, among others by Fujinami and Inamoto in Japan, by Teutschlaender in Germany, by Pentimalli in Italy, and by Begg in England. The two tumours discovered by Begg have an especial interest. The first, an endothelioma, was found *post mortem* in the region of the ovary in a White Leghorn hen. This tumour grows as a soft white mass composed of cells irregular in shape and presenting an indefinite alveolar arrangement. Metastasis formation is common, occurring especially in the liver and in the lymph nodes—which represent the remains of the thymus—in the neck. The second tumour is a fibro-sarcoma, which in its consistence, slow rate of growth and microscopic structure was in the earlier transplantations a typical fibroma. It has now accelerated its rate of growth, become softer and has proved to be filterable. The filtrates obtained from these tumours, as was found to be the case in the tumours studied by Rous, infect only fowls and no other species of animal. The filtrate of each tumour causes the formation of its own type of tumour and of no other. The 'agents' (the non-committal term used by Rous) are thus species-specific and cell-specific and would, therefore, appear to be different from each other.

Persistent efforts were made by Rous and Murphy to discover the nature of the 'agents' which cause these filterable tumours. The fact that they are reproduced indefinitely in the infected—the sarcoma—cells, that they are destroyed by antiseptics such as phenol, mercuric chloride, alcohol and chloroform, and that immune sera which operate in conjunction with complement can be prepared against them, led to the tentative conclusion that they are of the same nature as the filterable viruses found in so many infective fevers. The final proof that the 'agents' are living organisms depends upon their successful cultivation in the absence of living tissue and the reproduction of characteristic tumours with remote subcultures. All attempts to achieve this result have failed, just as similar attempts to cultivate other admitted viruses have failed. In spite of the close similarities which exist between the 'agents' which cause tumour formation in fowls and the 'agents' which give rise to acute specific fevers in other animals—e.g. foot-and-mouth disease of cattle—some authorities are reluctant to admit the tumour 'agents' as members of the virus class of micro-organisms. A discussion of the reasons for this reluctance belongs properly to text-books of pathology, but they are largely derived from the prevailing biological conception of the origin of cancer.

Of all these filterable fowl tumours the Rockefeller Institute tumour No. 1 has been most extensively studied. It has been found that filtrates are not constantly active and that wide variations in potency occur. Slow-growing, firm, yellow tumours usually yield inactive or barely active filtrates (Gye and Andrewes); rapidly growing, grey, necrotic forms give intensely active filtrates, the maximum potency to be expected being such that 0·001 c.cm. will induce tumour formation within 14 to 20 days.

Between these two extremes every grade of activity is observed, a rough correspondence—rough perhaps because of the crudity of filtration processes—existing between the growth energy of the tumour cells and the potency of the cell-free extracts. It has been found by Gye and Purdy that when the tumour cells are extracted with 5 per cent. or with saturated salt solution the filtrate obtained is more active than a filtrate made by extracting with normal saline.

A useful method of preparing tumour extracts consists in suspending tissue in broth or in serum broth and incubating at 37° C. for 1 to 5 days ; such extracts—diffusates—are thick and viscous and cannot be filtered. Diffusates in saline are relatively inert.

Filter candles absorb a variable but always large percentage of the active material. A coarse filter such as the Chamberland L1 candle absorbs approximately 80 per cent. of the virus, this figure being obtained by comparing the activity of an extract from which all cells have been deposited by spinning, with that of an L1 filtrate of the same material. Closer filters, such as Chamberland L2 or the Mandler or Berkefeld types, absorb still larger amounts.

The tumours induced by the action of cell-free filtrates almost always appear within four weeks after injection ; it is rare for a tumour to be delayed beyond six weeks. Young chickens are more sensitive to the virus than adult fowls, but variations in sensitiveness are found in both young and old. A chicken observed to resist an inoculation of a moderately active filtrate on one occasion, often responds to a later inoculation of a more active extract by the formation of a rapidly growing tumour.

The tumour ' agent ' is very sensitive to the effect of heat. When small volumes—3 c.cm.—of active filtrate are kept for 15 minutes at a temperature of 55° C. the tumour-producing power is lost absolutely. Feebly active filtrates are unable to withstand a temperature of 50° C. for 15 minutes (Gye).

The ' agent ' is similarly unstable at body temperature. Incubation of active filtrates at 37° C. leads rapidly—usually within the space of three hours—to the formation of an opalescence which later shows as a definite precipitate. Accompanying this change is a corresponding loss of infective power, which may disappear completely in several hours and which rarely remains after 24 hours' incubation. The changes which occur may be checked by adding HCN to the filtrate before incubation. A final strength of 1 : 4000 HCN preserves the activity of a filtrate for 2 to 3 days at 37° C. (Gye and Purdy). It is probable that tissue oxidases derived from tumour cells are responsible for the oxidative changes which lead to precipitations and to loss of infective power. Likewise, neutral cysteine in a dilution of 1 in 1,000 preserves infectivity (Mueller, 1928). It appears from these observations that free oxygen is detrimental to the life of the infective agent.

The action of antiseptics upon the tumour agent has been studied extensively by Gye and Purdy, who have found that it is not possible constantly to inactivate a cell-free extract with either chloroform or acriflavine or dilute 0·4 per cent. phenol. Moderatively active filtrates are relatively easily inactivated but the most active extracts resist antiseptics if precautions are taken to avoid the formation of precipitates. Acriflavine is a more active antiseptic in the presence of fresh horse-serum than in the presence of heated or old horse-serum, both towards the agent of the Rous Sarcoma No. 1 and towards the virus of bovine pleuropneumonia. This close similarity of action of acriflavine upon the invisible and subtle tumour agent and upon a visible and cultivable virus is strong evidence that the tumour agent belongs to the virus group of disease-producing micro-organisms.

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CHAPTER XXI. MUMPS.

BY M. H. GORDON (ST. BARTHOLOMEW'S HOSPITAL, LONDON).

MUMPS or epidemic parotitis is an acute specific fever usually benign, but exceptionally complicated by orchitis, meningitis, pancreatitis, ovaritis, &c. Histological examination of the parotid gland from cases of mumps reveals the presence of acute interstitial parotitis. The blood count shows as a rule an absolute increase in the lymphocytes. According to Monod, even mild cases may show increased lymphocytes in the cerebrospinal fluid.

The disease is usually contracted from a previous case, and has an incubation period of 14 to 21 days. Mumps is met with all over the world. In this country it is more frequent in cold weather. The deaths annually ascribed to mumps in England and Wales do not exceed 2 or 3 per million living.

Early efforts to define the pathogenic agent of mumps by the ordinary bacteriological methods were unsuccessful. Later work, however, has shown that the disease is communicable to animals, and that the causal agent is filterable. The progress of research on this matter is briefly as follows: In 1908, Granata found that the sterile filtrate obtained by passing saliva from patients in the acute stage of mumps through the Berkefeld filter produced, when injected intravenously into rabbits, a fever of three days' duration. Comparable material from normal persons had no such effect. When injected into the parotid gland of the rabbit or subcutaneously, filtered saliva from cases of mumps gave rise to a swelling of the parotids that did not proceed to suppuration. In 1913, Nicolle and Conseil found that fluid obtained by puncturing the parotid gland during mumps, although free of visible micro-organisms, produced, on intraperitoneal injection into three Bonnet monkeys, after an incubation period of 16 to 30 days, a febrile illness of 4 to 6 days' duration. One of these monkeys developed, together with the pyrexia, a swelling of the left parotid. The fever appeared to be specific, since one attack conferred immunity. The blood of one of the monkeys was examined during the attack and found to show a lymphocytosis.

In 1914, Gordon, who was investigating buccopharyngeal secretion for the presence of poliomyelitis virus, undertook some control observations with similar material from cases of mumps. A saline gargle from each of ten cases in the acute stage of mumps was freed of bacteria by passage through a Berkefeld filter, and a portion of the filtrate of each injected intracerebrally into a separate monkey (*M. rhesus* and *M. cynomolgus*).

On the third or fourth day, five of these monkeys developed symptoms of meningitis, and four of them died. In one of these animals the cerebrospinal fluid during life was found to be free of bacteria, and to contain 1,500 cells per c.mm., of which 82 per cent. were lymphocytes. The blood and cerebrospinal fluid of the four monkeys that died were free of bacteria, and in all a lymphocytic meningitis was present. Microscopically the substance of the brain and cord was congested. Perivascular infiltration was absent except in one case, where a focus of it was found at one spot in the cortex, but not elsewhere. A proportion of the nerve-cells of both cortex and cord showed pronounced degenerative changes leading in some cases to almost complete lysis. The parotid glands of three of the four monkeys were normal, but those of the fourth, that had survived longest, showed the lesions of acute interstitial parotitis.

When the same filtrate was injected into one monkey intracerebrally, and into another intravenously and intraperitoneally, the former developed meningitis on the fourth day and succumbed, the other monkey was well till the eleventh day, when it became ill without meningeal symptoms and developed a swelling of both parotids. Heating the filtrate to 55° C. for one hour destroyed its capacity to produce meningitis on intracerebral inoculation. Transference experiments were unsuccessful.

In 1916 the experimental study of mumps was resumed by Wollstein, who injected the filtered saliva of children in the acute stage of mumps into the parotid and testis of cats. After an interval of 5 to 9 days swelling and tenderness appeared in both organs: the testicle being the more affected of the two. A rise of temperature was found to precede the glandular swellings, but all the changes reached their maximum at the same time. An absolute increase in the lymphocyte count followed a preliminary neutrophile leucocytosis. An important advance was made by Wollstein, who showed that the disease could be transferred in series: the best time to effect passage being from the fourteenth to seventeenth day, when the experimental disease was at its height. After the third or fourth passage the virus became more active, but on further passages it deteriorated. Histological examination of the affected parotids revealed in a proportion of the cases the lesions of acute interstitial parotitis. The chief change found in the testis was an acute degeneration of the tubular epithelium. In a second communication, Wollstein found (as Gordon had done) that buccal secretion of adults during an attack of mumps was more pathogenic than that obtained from children. She now succeeded in showing that by the sixth day of the attack the salivary filtrate was less active, and by the ninth day it became inert. A further new fact established by Wollstein in the second outbreak was the presence of the virus in the blood of cases with severe constitutional symptoms. In a third paper, Wollstein showed that the salivary filtrate of children in the acute stage of mumps produced meningitis in cats when introduced into the cisterna magna, and that the cerebrospinal fluid of the affected animal would convey the disease to others.

The most recent contribution to the study of the ætiology of mumps is that of Kermorgant, who substituted the centrifuge for filtration in order to concentrate the virus in the buccal secretion removed at the onset of mumps. Having found that 0·1 c.cm. of the salivary deposit reproduced the disease in all of three monkeys when injected into the parotid gland, he closely examined the infective material, and found a spirochæte that he was able to grow anaerobically in symbiosis with a Gram-negative bacterium of the saliva. The inoculation of 0·1 c.cm. of such a mixed culture reproduced the disease in the same manner that the salivary secretion had done, and he found that this result could be obtained after 'a sojourn of 10 months in the incubator (29° C.) and 14 subcultures'. Inoculation under identical conditions of the bacterium alone gave negative results. On passing the mixed cultures through a Chamberland L2 or L3 filter, the bacterium was removed, but the filtrate was still infective to monkeys; and on cultivating the filtrate in symbiosis with the bacterium he found that in about a month the spirochæte appeared in the culture. Kermorgant attributes the passage of the spirochæte through the filter to its granular phase. He failed to demonstrate the spirochæte in the tissues of animals infected with mumps, but succeeded in cultivating it from this source by symbiosis with the bacterium. Finally Kermorgant found both agglutinin and lysin for his spirochæte in the serum of mumps convalescents. He concludes that mumps is a spirochætosis.

From the evidence adduced it is clear that the pathogenic agent of mumps is filterable. The work of Wollstein in particular shows that the agent here in question is capable of producing all of the essential lesions of mumps. The investigation of Kermorgant is of special interest because of his success in cultivating the virus. The identity, however, of the spirochæte of Kermorgant with the pathogenic principle of mumps is not yet beyond doubt. No one has yet successfully demonstrated this organism for certain either in the parotid, or in the testis, or in the cerebrospinal fluid during mumps (Sabrazes and Broustet); and arsenical preparations have had but little effect on the disease (Molinelli). So far, but little attention has been given to the serology of mumps.

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CHAPTER XXII. MEASLES.

BY J. E. McCARTNEY (METROPOLITAN ASYLUMS BOARD).

AN outstanding feature of measles is its extreme infectivity, and a susceptible person exposed to the infection almost always contracts the disease. The causal agent is presumably air-borne, and is present in the nasopharyngeal secretion, but its precise nature is not yet known. Measles is essentially a human disease and never occurs spontaneously in animals.

Experiments on Human Beings.

The first transmission experiments were made in the middle of the eighteenth century by a Scots physician named Home (1759). In 1905, Hektoen showed conclusively that the causative agent of measles is present in the blood-stream, and that when susceptible individuals are inoculated with blood taken immediately after the appearance of the rash, typical measles supervenes. These experiments were repeated and confirmed by Degkwitz (1927).

Experiments on Animals.

Experiments on human beings cannot be undertaken extensively, and numerous workers have tried to communicate the disease to animals. Josias (1898) and Anderson and Goldberger (1911[1]) reported the successful transmission of measles to monkeys. Nicolle and Conseil (1911) also reported similar experiments, but no definite conclusions can be drawn from their work.

Other workers (Hektoen and Eggers, 1911; Tunnicliff, 1912; Lucas and Prizer, 1912; Jurgelunas, 1914; Sellards and Wentworth, 1919; Sellards, 1919) have been unsuccessful in transmission experiments from man to monkeys, and either present little or no evidence of infection, or else have reported completely negative results. The most convincing work on the experimental transmission of measles to monkeys was made by Blake and Trask (1921). These observers produced a condition resembling measles in 8 out of 10 monkeys infected in the upper respiratory passages with human nasopharyngeal washings taken in the pre-eruptive or eruptive stage. Berkefeld filtrates of the same material produced similar results. The infection was carried successfully through six passages by intratracheal injections of tissue emulsions in saline made from animals killed shortly after the appearance of the exanthem. Experimental measles was also transmitted from monkey to monkey by the intravenous injection of citrated whole blood. In these experiments cultures of the transmitted blood were made, and none showed growth. These results are of significance when considering the claims recently made, that certain green-producing cocci are the cause of measles. In Blake and Trask's experiments, measles in monkeys are characterized by a relatively constant group of symptoms which resemble closely the disease in man. The histological findings in the skin and buccal mucous membrane of the experimental monkeys are exactly like those found in human measles by Ewing (1909), and Mallory and Medlar (1920). Monkeys recovered from experimental measles are immune to further infection from measles, irrespective of whether the virus is the same strain as that of the original infection or from another patient.

With regard to animals other than monkeys, Duval and d'Aunoy (1922) claim to have transmitted measles by means of human blood to rabbits and guinea-pigs, while Hardie (1921), Nevin and Bittman (1921) and Grund (1922), state that they have transmitted the disease to rabbits. An analysis of their protocols, however, shows that their conclusions are not warranted, and that no definite evidence of the experimental transmission to rabbits and guinea-pigs was obtained. In contrast with the above results, Purdy (1925) was unable to find any evidence of transmission when measles material was inoculated into rabbits, and the present writer has failed to transmit measles by means of human nasopharyngeal secretions (filtered and unfiltered), and whole blood, to rabbits, guinea-pigs, rats, mice, ferrets, hamsters, gerbilles and pigeons. From results obtained up to the present it thus would appear that the monkey is the only animal to which measles can be transmitted.

Immunity in Human Measles.

It is an undoubted clinical fact that an attack of measles almost invariably renders the individual immune for life, and it seems evident that a powerful immunity is produced.

Passive immunity. Nicolle and Conseil (1918) first published results on the injection of serum from convalescents to immunize children who had been in contact with the disease. Park and Zingher (Zingher, 1924) in 1916, injected 41 children recently exposed to measles. Since that time numerous observers have recorded similar results and all agree as to the efficiency of human convalescent serum if taken during convalescence and injected in adequate amounts shortly after exposure to infection. Others use the serum, as suggested by Debré and Ravina (1923), to modify the disease to a very mild form, thereby inducing an active immunity. The immunity conferred by the serum is, of course, only passive and lasts from two to four weeks. The blood should be drawn from convalescents shortly after the temperature returns to normal, 7 to 11 days being advocated by some observers.

Active immunity. Hiraishi and Okamoto (1921) endeavoured to protect against measles by injecting very small quantities of diluted blood taken from a patient in the catarrhal stage. No convincing results, however, were obtained. Debré and his colleagues (1926) have given two injections of diluted measles blood in quantities of 1/800 and 1/400 c.cm. respectively. Their results are promising, but at present the work is not sufficiently developed for any conclusions, favourable or otherwise, to be given.

The Various Organisms claimed to be the Causal Agent of Measles.

Caronia's organism. Caronia (1923) reported that in Tarozzi-Noguchi media under anaerobic conditions there can be cultivated, from the blood and secretions of measles patients, a small Gram-negative organism, round in shape and arranged in pairs like diplococci. His claim that these so-called organisms were the causal agent of measles has been conclusively disproved (McCartney, 1927; Meyer, 1925, 1926; Takaki, 1926; Zlatogoroff, Burowa and Nasledyschewa, 1928; Tunnicliff, 1917, 1918, 1919, 1925).

The green-producing cocci. Tunnicliff (1917, &c.), Ferry and Fisher (1926), Hibbard and Duval (1926), Cary and Day (1927), have isolated a small, round, sometimes flattened diplococcus occurring in short chains or clumps, which produces small green colonies on blood-agar. Although differences are mentioned by the various workers, e.g. Tunnicliff says it only grows under anaerobic conditions when first isolated, while Ferry and Fisher describe it as an aerobe, they appear to have dealt with essentially the same organism.

Tunnicliff's organism will only develop in primary cultures on blood-agar under strict anaerobic conditions. In subculture it grows aerobically. It may then be grown on ordinary agar or in broth, although the growth is scanty. It ferments glucose and saccharose, and all strains but one ferment maltose. Mannitol and lactose are occasionally fermented, but never inulin, salicin or raffinose. The green-producing diplococcus is non-soluble in bile and does not liquefy gelatin. The organism is Gram-positive. Recently isolated strains pass a Berkefeld N filter and the organism resists drying for 24 hours, but not 48. It is easily killed by heat (45 minutes at 57° C.) but survives for 14 days at 0 to 2° C. and for 2 months in 50 per cent. glycerin.

Tunnicliff and her co-workers (1918) state that opsonins and complement-fixing bodies to the micro-organism can be demonstrated in human convalescent serum. She has also described a skin reaction for measles, analogous to the Dick test in scarlet fever, but as the organism does not produce a strong extracellular toxin, killed whole-broth cultures, suitably diluted (about 1 in 40), are used for the test. Positive reactions were observed in persons with no history of measles, but not in those convalescent from the disease or previously affected.

An immune serum to the Tunnicliff coccus has been produced from goats and favourable results have been claimed for it when it is injected prophylactically (Tunnicliff and Hoyne, 1926[1 & 2]; Hoyne and Gasul, 1926; Hoyne and Peacock, 1928; Halpern, 1928).

Ferry and Fisher (1926) isolated an organism which they named the '*Streptococcus morbilli*', the toxin of which they claim is specific to measles.

It differs somewhat from the Tunnicliff organism, inasmuch as it grows luxuriantly in the presence of oxygen and produces a powerful toxin. Their earlier statement that the toxin was specific for measles is not warranted by an examination of their protocols. Later, Ferry and his colleagues (1928) carried out more extended observations, using immune horse-serum made from the coccus. It was given prophylactically in doses of 20 c.cm. and their results show that protection was afforded in 78 per cent. of cases, but with smaller doses the protection was much less. It must be remembered, however, that equally good prophylactic results have been obtained with normal horse-serum or antidiphtheritic serum (Pontano and Corradi, 1927).

The relationship of the various green-producing cocci to measles has been criticized and doubted by several workers. Park, Williams and Wilson (1927) isolated similar cocci and showed by fermentation and agglutination tests that there was a great diversity of types, and that they could not differentiate immune and non-immune persons by intracutaneous inoculation of toxin. Ferry and Noble (1929) examined green-producing cocci isolated by other workers and compared the strains with their own organism. They found that the different organisms were not similar and that they varied morphologically, culturally and serologically. Long and Cornwell (1927) made blood cultures from early cases of measles. They were unable to find green-producing cocci in 47 cultures from 26 cases.

Smith (1929) examined cultures of Tunnicliff's and of Hibbard and Duval's organisms, and found them indistinguishable from the green-producing cocci obtained from non-measles cases. Similar results were obtained by Bradford (1929).

The Use of Animal Sera.

Immune sera have been made from Tunnicliff's coccus, both from goats and horses, and some of the results with goat's serum have already been mentioned, while Ferry and others (1928) has reported his observations with immune horse-serum made with '*Streptococcus morbilli*'. In addition, Degkwitz (1926[1 & 2]) has produced from sheep a serum which, he claims, acts similarly to human convalescent serum. The reports on the use of this serum have not been satisfactory (Kochmann, 1926; Kaupe, 1926; Seligmann and others, 1926; Zoepffel, 1927; Leiner, 1929). The results of numerous observers indicate that none of the animal sera protect against measles. For example, Gunn (1928) has carried out a series of tests in which human convalescent serum, Tunnicliff's serum, Ferry and Fisher's serum, and Degkwitz serum, were compared together. In all, 112 individuals were injected and the degree of protection obtained was as follows:

Convalescent serum	95·7 per cent.
Tunnicliff's serum	42·9 ,,
Ferry and Fisher's serum	0·0 ,,
Degkwitz's serum	40·0 ,,
Control group (no serum)	21·7 ,,

In connection with these results it must be borne in mind that normal horse-serum appears to afford a certain degree of protection (Pontano and Corradi, 1927).

Attempts to Cultivate the Virus of Measles.

Degkwitz (1927) has carried out experiments on the transmissibility and cultivability of measles virus, using susceptible human beings as a test for the presence of infection. Attempts were made to cultivate the virus in association with living cells. At first, tissue cultures were employed, but later, pneumococci, streptococci and Tunnicliff's coccus, as these organisms are found regularly in measles cases. The growth of these accessory bacteria must be controlled, as overgrowth kills the virus. The culture medium consists of human blood-plasma and buffered saline solution made according to a special technique. The primary inoculum is blood from an early case of measles, and subcultures are made every 8 to 9 days. When the symbiotic organisms are too numerous, the cultures are filtered through a Berkefeld filter and the filtrate, which contains the virus, is transferred to a fresh set of cultures. After the second subculture, sheep's plasma is substituted gradually for human plasma, so that at the sixth subculture sheep-plasma alone is used.

Virus cultures have been carried through 16 to 18 generations, and Degkwitz states that subcutaneous inoculations give rise to specific reactions in susceptible human beings. Typical measles, however, is not produced, but between the tenth and fourteenth day after injection, there is a rise of temperature, conjunctivitis, rhinitis, bronchitis, and stomatitis. A typical measles rash has never occurred, but sometimes a bluish-red flush is seen. The reactions are thought by Degkwitz to be specific, because control cultures with symbiotic organisms but without virus gave no results. Individuals treated with virus cultures were immune to subsequent infection with infective blood, while those who received control material reacted to measles blood inoculation. The result of the injection of monkeys with virus cultures was, however, unsatisfactory. Degkwitz (1928) explains this by stating that a high percentage of monkeys are immune to measles inoculation. The serum of the injected monkeys was stated to protect susceptible human beings in the same manner as human convalescent serum (Degkwitz, 1928). Degkwitz, therefore, claims that measles is a filterable virus, which can be grown in culture media in association with organisms normally found in the throat, such as streptococci and pneumococci.

Summary.

In spite of the large amount of work done on measles there is little definitely known about the causal agent. The available evidence indicates that it is a filter-passer and not a recognizable organism. It may be regarded as proved that the virus is present in the nasopharyngeal secretions and in the blood-stream. The infection can be communicated in a proportion of cases to monkeys, in which it produces a disease comparable with the human condition, and the serum of recovered animals is stated to protect susceptible individuals in a manner similar to human convalescent serum. There is no evidence to show that measles can be transmitted to any of the lower animals apart from monkeys.

The claims of Caronia for his organisms have not been substantiated. In spite of the large amount of literature on the green-producing cocci there is no evidence to show that they have any definite ætiological relationship with the disease, or that they are essentially different from the ordinary green-producing cocci found in non-measles cases. Injections of these organisms in human beings with the production of typical measles which can be transmitted to other individuals is the only crucial test by which their causal relationship can be proved. This has not been forthcoming. The animal sera produced from the green-producing cocci have failed to show any immunological relationship to measles, as evidenced by their inability to protect susceptible persons from infection. Any slight protection claimed for them is no more than can be secured by the use of normal horse-serum. In fact, the use of these animal sera may be detrimental, for not only do they fail to prevent the onset of measles, but the subsequent serum rash may seriously aggravate the condition.

With regard to the claims of Degkwitz regarding the culture of the measles virus, it must be emphasized that the work must be repeated carefully and extensively with full controls before any conclusion can be drawn. Measles is an extremely infectious disease and it is difficult to guard against the natural spontaneous infection. Degkwitz has not produced the definite and typical disease in monkeys, and a careful examination of his protocols shows that he failed to induce typical measles in human beings by the inoculation of his cultures. He produces certain evidence that they were immune to subsequent injections of infective blood, but as there were only two individuals injected, no conclusions either way can be drawn from his results. The prophylactic use of Degkwitz's animal serum, presumably made from the virus cultures, has not shown the slightest evidence that immune bodies to the measles virus are present, and the precise evaluation of Degkwitz's claims cannot be made until further and more extensive investigations are made. From our clinical knowledge of the properties of the virus it would appear that the lines of research pursued by Degkwitz may yield results.

Experimental work has been handicapped by the failure to find an animal in which typical measles can be produced, and which can be obtained easily in numbers. The only criterion of the presence of measles virus is its ability to produce the typical disease when injected. Human beings appear to be the only suitable experimental subjects, and it must be recognized that more extensive human transmission experiments are necessary if further knowledge of the measles virus is to be gained.

German Measles.

Nothing is known of the precise nature of the causal agent of this disease.

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CHAPTER XXIII. CATTLE PLAGUE.

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CATTLE PLAGUE (*French :* Peste bovine, typhus contagieux ; *German :* Rinderpest ; *Italian :* Peste bovina) is an acute contagious febrile disease affecting chiefly bovines and presumably due to a specific ultramicroscopic virus.

History.

In the old world from the earliest historical times serious epizootics amongst cattle have been repeatedly recorded, and, although these early records naturally do not differentiate cattle plague from foot-and-mouth disease, pleuro-pneumonia, anthrax and other maladies, the later accounts leave no doubt as to the enormous losses caused by the disease. Cattle plague appears to have always been endemic in large areas of Asia, especially the region of the Central Steppes, and it was from these centres that the periodic invasions of Europe originated. Gamgee (1866) states that it is probable that a period of fifty years rarely elapsed without a visitation of ' the murrain of the East ' to some part of the European continent. In Europe in the past the disease commonly followed in the train of moving armies ; thus it followed the Austrian troops into Lombardy in 1793, at the end of which year it entered Piedmont, and gave rise to an epidemic which in three years is said to have killed between three and four million head of cattle in Italy. During the course of the eighteenth century it is estimated that some thirty million cattle died from cattle plague (Ostertag, 1928).

In more recent times the introduction of the disease into Cape Colony and the Transvaal in 1897 caused the loss of over two million cattle in two years. The American continent has been fortunate in this respect, and remained free from the disease until 1921, when it was introduced into Brazil. The last great epidemic in England was in 1865, and was responsible for the death of some 500,000 cattle in 1½ years. A small outbreak occurred in 1877, but this was promptly dealt with and did not cause any serious loss. It was during the 1865 outbreak in England that Dr. Burdon Sanderson, in the work of the Royal Commission appointed to study the disease, first definitely established the infectivity of the blood and of the mucous discharges of animals suffering from cattle plague.

The earlier history of the disease is very fully dealt with by Gamgee (1866), and particulars of more recent outbreaks are summarized by Hutyra and Marek (1922).

Susceptibility of Different Species.

Cattle plague is primarily a disease of bovines and all races of cattle are susceptible, although the degree of susceptibility varies somewhat widely. It has been repeatedly observed that hill cattle are more susceptible and less easily immunized than animals from the neighbouring plains. This has been especially noted in India, Russia and the Philippines. It seems probable, however, that the greater degree of immunity amongst the latter class of animals may be rather inherited than natural, as in a country where the disease is endemic cattle in the hill districts are more likely to escape infection than those in the plains. The buffalo is susceptible to the disease, but commonly contracts it in a mild form, often showing no symptoms beyond a slight rise of temperature, although in the Philippines the carabao (water-buffalo) suffers severely and shows a high mortality. The susceptibility of sheep and goats appears to vary greatly in different races, and it is still uncertain if these animals play any part in the natural spread of the disease. They can be artificially infected by the injection of virulent blood, and usually show no symptoms beyond a slight rise of temperature, although Schein (1917) in Indo-China found that the local goats were highly susceptible, showing a mortality of 87 per cent. after injection with virulent blood, and McKinley (1928) was able to infect the Philippine goat with a dose as small as 1/5,000 c.cm. of virulent blood, the animals showing mild symptoms with some fever, but as a rule recovering. A heavy mortality has been recorded amongst various species of antelope, and in South Africa the disease was undoubtedly spread over large areas by means of big game. Cases have also been described in camels and dromedaries. The pig does not usually contract the disease naturally, but some races can be artificially infected. Other species appear to be immune and attempts to transmit the disease to cats, dogs, monkeys, rabbits, guinea-pigs, fowls, rats and mice have invariably given negative results. Man also is completely immune.

Symptoms.

The incubation period is commonly given as from 3 to 9 days, but when animals are artificially infected by swabbing the nostrils with the nasal secretions of sick animals, so as to simulate more closely the conditions of natural infection, and at the same time to be sure of the exact time of infection, the first rise of temperature usually occurs after 3 or 4 days, less frequently after 5 days, and only occasionally after a longer interval. The course of the temperature in an animal so infected is shown in Chart 1.

In cattle infected by the subcutaneous injection of virulent blood (1 c.cm.) the incubation period is somewhat shorter, being remarkably constant and averaging 72 hours. Of 637 Cyprus cattle inoculated with virulent blood 15 per cent. showed the first rise of temperature after 2 days, 69 per cent. after 3 days, 15·5 per cent. after 4 days, and 1·5 per cent. after 5 days (Todd, 1912). The typical course of the temperature after the subcutaneous injection of virulent blood is shown in Chart 2, which is a composite chart obtained by averaging the temperatures of 20 consecutive Cyprus animals, each of which had received 1 c.cm. of virulent blood.

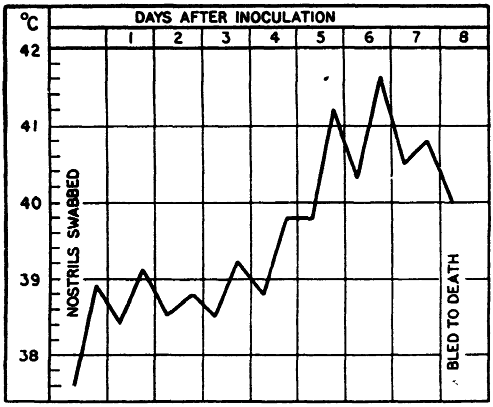

CHART 1. Temperature chart of a Cyprus bull infected by swabbing the nostrils with nasal mucus from an animal on the fifth day of cattle plague.

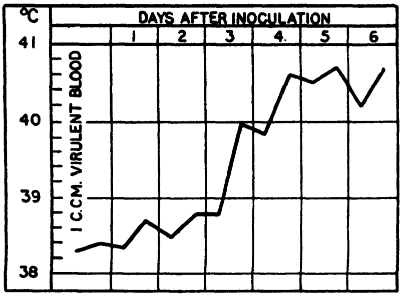

CHART 2. Composite chart of the temperatures of 20 Cyprus cattle inoculated subcutaneously with virulent cattle-plague blood.

The above statements refer to highly susceptible animals which have received an amount of the virus representing many times the minimal infecting dose ; if the dose is materially diminished the incubation period may be longer. In countries where cattle plague is endemic and a certain amount of immunity is present longer incubation periods are commonly observed.

The beginning of the fever is accompanied by twitchings and general restlessness, with loss of appetite and cessation of rumination. As the disease progresses with increasing temperature the animal grinds its teeth, arches its back and draws its hind legs together under the body ; there is a glairy discharge from the eyes and nose with redness of the visible mucous membranes. Later the restlessness becomes more marked and the animal shows signs of abdominal pain, severe diarrhœa sets in and the discharges are often fœtid and tinged with blood. The symptoms rapidly increase in severity, with acute diarrhœa, weakness, and coldness of the extremities; death commonly taking place after some 4 to 7 days in acute cases. In milch cattle the secretion of milk ceases and pregnant animals usually abort. The disease is particularly fatal in cattle infected with tubercle, and according to Nicolle and Adil Bey (1899) these animals rarely recover.

The mortality varies greatly, but in areas previously free from the disease is very high, being commonly from 80 to 90 per cent. and even higher. In countries where the disease is endemic the mortality is much lower and many animals contract mild attacks.

An excellent account of cattle plague in susceptible cattle is given by Gamgee (1866) who had the opportunity of studying the disease in a country known to have been free from the plague for over a hundred years.

Anatomical Lesions.

The most important changes are seen in the mucous membranes, particularly those of the alimentary canal. The mucous membrane of the mouth is excoriated particularly on the lips and gums, and the mucous surface of the abomasum is swollen, intensely red, and often shows minute superficial ulcers. Claret-coloured patches of discoloration, apparently due to submucous extravasation, may be present. The intestine is more or less inflamed throughout its length with intensely injected mucous membranes whose epithelium is desquamating, and ecchymoses may be present. The lymphatic glands are usually red and swollen and may also show ecchymoses. There is marked congestion of the lungs with patches of broncho-pneumonia. The gall-bladder is often distended with dark green or yellowish bile sometimes tinged with blood. The other organs do not show marked naked-eye changes, and the spleen in uncomplicated cases is not enlarged.

The changes taking place in the blood during the disease do not appear to have been adequately investigated, but Curasson (1921) states that it is uncertain if the leucocyte count undergoes any marked changes save that in some instances there is a slight increase in the total number of white cells during the first few days, and then a slow decrease, which persists while the blood remains virulent.

The septicæmic character of the disease and the absence of any symptom or pathological lesion which is specifically characteristic may render the diagnosis of cattle plague a matter of some difficulty, particularly if the diagnosis has to be based on the examination of a single case and apart from a general outbreak. In cases where it is important to establish the diagnosis, such as the occurrence of suspected cases in a country hitherto free from the disease, the inoculation of susceptible cattle is the only satisfactory method. This is best done by injecting one animal subcutaneously with several c.cm. of the blood and by infecting a second animal through the mucous membranes, preferably of the vagina, with nasal discharge—or better with scrapings from the mucous membrane of the abomasum. Examination of the temperature charts of the experimental animals together with the clinical symptoms shown will usually allow of a definite diagnosis being made. In order to exclude the possibility of hæmorrhagic septicæmia it is a wise precaution at the same time to inoculate a rabbit with some of the blood, as this animal, while highly susceptible to the causal organism of bovine septicæmia, is unaffected by cattle plague.

Ætiology.

Although the causal agent of cattle plague is present in a somewhat high concentration in the blood, secretions, and excretions of sick animals during the febrile stage of the disease, its exact nature is as yet unknown, but, in view of its invisibility, alleged filterability, and general behaviour it is commonly classed with the ultramicroscopic viruses.

The Virus.

Filterability. The results of filtration experiments are somewhat conflicting; the earlier workers—Semmer (1896), Nencki, Sieber and Wyznikiewicz (1898), and Kolle (1898)—all found that the virus did not pass bacterial filters, but Nicolle and Adil Bey (1899, 1902) were able to infect animals with the Berkefeld filtrates from peritoneal washings and from emulsions of the brain and stools by the use of filters whose walls had been thinned by shaving down the candle so as to reduce its diameter from 25 mm. to 21 mm. The candles so treated did not allow the passage of fowl-cholera bacilli used as a control.

Nicolle and Adil Bey were unable to infect animals with filtrates from Chamberland candles, but state that some of the cattle which received these filtrates were subsequently found to be immune. Yersin (1904), working with peritoneal washings, found that the virus passed the Chamberland F, but not the B candle. Ruediger (1908 and 1909), on filtering virulent blood through Berkefeld candles (V, N and W), obtained non-virulent filtrates, but, when peritoneal washings were used, the virus was found to pass all of these filters. Even from peritoneal washings, however, it would not pass the Chamberland F, although the most porous candles of this grade were selected. Filtering diluted virulent blood, Memmo, Martoglio and Adani (1904), obtained positive results with Berkefeld, but not with Chamberland candles, and Woolley (1906) found that the virus may or may not pass through the Chamberland filter according to the conditions of the experiment. Todd (1907) was unable to infect with 50 c.cm. (representing 10 c.cm. of whole blood) of the filtrate from coarse Berkefeld candles. Hornby (1928) has recently repeated these experiments with coarse Berkefeld filters and found that, while 40 c.cm. of the filtrate did not infect, 80 c.cm. caused the disease, but he does not state whether the candles used were tested for their impermeability to ordinary bacteria. Daubney (1928), working with standard coarse Berkefeld filters and also with Seitz filters, found that the filtrates from virulent blood were non-infective in doses corresponding roughly to 10,000 to 20,000 minimal infective doses of the whole blood. The balance of evidence thus seems to indicate that, while the virus as it exists in peritoneal washings and emulsions of certain organs may be capable of passing a Berkefeld candle, similar filtrates from virulent blood are non-infective—except possibly in very large doses.

Intracellular location of the virus. It appears probable that the discordant results in filtration experiments obtained by different workers are to be attributed to the fact that the virus does not normally exist in a state of freedom in the body fluids, but that it is mainly intracellular. Semmer (1896) suggested that the causal agent was attached to the white blood corpuscles, but that after the breaking down of these it could exist free in the serum, and Kolle (1899) found that if citrated virulent blood was spun in the centrifuge for from 20 to 30 minutes at 2,500 to 3,000 revolutions per minute the supernatant plasma was non-virulent, while the deposit was highly infective. Kolle's results were confirmed by Todd and White (1914). Nicolle and Adil Bey (1902) also expressed the opinion that the virus was probably intraleucocytic.

The connection of the virus with the white cells of the blood was demonstrated by Todd and White (1914), who, by fractional centrifuging of the cellular deposit of virulent blood separated the white and the red blood corpuscles in a state of relative purity, and found the concentration of the virus in the leucocytic layer so obtained to be about one hundred times as great as in the red cell layer, although the separation of the two types of cells was naturally not complete. These results have been confirmed by Schein (1917), who, working with goats in Indo-China, found that the virus in virulent blood is mainly contained in the leucocytes, although a small amount is also free in the plasma. He estimates that 1 c.cm. of complete virulent blood contains approximately 25,000 'microbes' or groups of 'microbes', and that 1 c.cm. of centrifuged citrated plasma contains 10 'microbes'. Hornby (1928), from centrifuging experiments and by freeing virulent blood from leucocytes by filtration through cotton-wool, has obtained similar results, finding that more than 98 per cent., and possibly 99 per cent., of the virus of cattle-plague blood is attached to the leucocytes. Daubney (1928) has similarly shown that virus is removed from virulent blood by a filtration method which removes the leucocytes. He was, moreover, unable to obtain any evidence that the virus could be detached from the leucocytes by washing with solutions of varying reactions. There is thus good evidence that the virus as it exists in the body is intimately connected with certain of the white cells found in the blood and it seems probable that it is actually intracellular.

Attempts to cultivate the virus have been generally unsuccessful. Boynton (1914), using a modification of Bass and John's medium, found that in two separate series of tubes after incubation under sterile paraffin at 40° C., the sixth transfer was infective—in one instance at a dilution corresponding to 1/2,800,000 c.cm. of the original blood, and he suggested that there was actual multiplication, but Minett (1923), who repeated Boynton's experiments, using the same technique, obtained negative results, except in one case where the factor of survival could not be ruled out. Daubney (1928) also made numerous attempts to cultivate the virus in Bass and John's and similar media, but in no case was any evidence of growth obtained.

Resistance of the virus to physical and chemical agents. All observers appear to agree that drying causes a rapid inactivation of the virus. Koch (1897), showed that the virus is very rapidly inactivated by drying and the same conclusion was arrived at by Nicolle and Adil Bey (1901), who found that an emulsion of virulent lymphatic glands lost its virulence on drying for two days *in vacuo,* and Rogers (1900) states that blood dried in sterilized Petri dishes (which process took about 24 hours) and then dissolved in saline was non-virulent. The virus in defibrinated virulent blood is inactivated by heating for half an hour at 50° C., but not at 45° C. (Baldrey, 1911).

The statements made as to the length of time for which cattle-plague blood remains virulent when kept *in vitro* at ordinary temperatures are very conflicting—varying from three days to several months, but most observers give few exact details as to the conditions under which the blood was kept—particularly as regards temperature. The question of the conservation of the virulence of infective blood is of some importance in view of the extensive use of such blood in the process of simultaneous inoculation, which has often to be carried out over large areas, and Stockman (1905) states that in the plains of India blood from a sick ox could not be depended on to retain its virulence for more than 48 hours, even when kept in the ice-chest, and says in a footnote that the experience in South Africa was the same. This is probably explained by the difficulty of maintaining a low temperature and of keeping the blood sterile under field conditions, as Todd and White (1914) showed that virulent blood kept at 0° C. in melting ice retains its virulence for at least a fortnight, and that at temperatures below freezing-point the virulence is maintained for much longer periods; citrated blood kept frozen solid at —6 to —7° C. is virulent for at least 22 weeks. It was noticed in these experiments that the incubation period of the disease in the injected cattle appeared to lengthen as the period for which the blood had been kept frozen increased.

The majority of the data found in the literature concerning the effect of various chemical agents on the virus are not sufficiently precise to be of value, as usually no details are given of the conditions under which the experiments were made; this is particularly the case as regards the reaction of the medium, as at the time when most of the earlier work was done the importance of the hydrogen-ion concentration was not appreciated.

All observers find that the virus is inactivated by the bile of normal cattle, and it is generally agreed that the virus is somewhat easily destroyed by glycerin. Semmer (1896), noticed that the virus of cattle plague, in contrast to that of vaccinia does not keep long in glycerin, and Koch (1897), who confirmed this, noted that the fact is remarkable, as most other infective materials, particularly small-pox vaccine, are not easily destroyed by glycerin.

Daubney (1928) found that an emulsion of virulent spleen pulp in Ringer's fluid was inactivated by treatment for 3½ hours at 37° C. with 10 per cent. chloroform ; the virus was also inactivated by the addition of 0·1 per cent. of formalin to a 1 in 4 emulsion of virulent spleen pulp and incubation at 37° C. for 72 hours.

Presence of the virus in the body fluids, organs, &c. As a general rule the presence of the virus in the blood appears to coincide with the period of fever, and Todd and White (1914), working with susceptible Cyprus cattle in Egypt found that, when these were inoculated subcutaneously with a small dose (1/50 c.cm.) of virulent blood, the blood of the animals remained non-infective for the first 72 hours, and usually became infective during the course of the succeeding 24 : the appearance of the infectivity of the blood coincided with the rise of temperature. On the seventh day after inoculation the amount of blood required to convey the disease by subcutaneous inoculation varied between 1/500 and 1/2,000 c.cm. This agrees with experience elsewhere, Schein (1915) and Daubney (1928) both finding that 1/1,000 c.cm. was generally infective, and Hornby and Hall (1928) giving the normally infective dose as 1/50 c.cm. to 1/5,000 c.cm. The infectivity of the blood appears normally to disappear with the subsidence of the temperature. At the height of the disease the nasal discharge, urine, fæces, aqueous humour, cerebrospinal fluid and lymph collected from the thoracic duct are all infective by subcutaneous injection as are also emulsions of the viscera and muscles. The infectivity of the nasal discharge and of scrapings from the mucous membrane of the fourth stomach is especially high.

The fact that the cerebrospinal fluid and aqueous humour—fluids containing no red blood corpuscles—are always infective was pointed out by Nicolle and Adil Bey (1902) as evidence that the red cell is not the site of the virus.

Possible Existence of Carriers.

Amongst cattle recovering from cattle plague a certain number of individuals are met with whose convalescence is slow, the animals remaining poor in condition with intermittent febrile attacks, cough, chronic vaginitis and other symptoms. Such animals if slaughtered commonly show patches of inflammatory congestion at various points in the alimentary canal with enlarged mesenteric glands, &c. These lesions are usually regarded as being due to secondary bacterial invasions of the tissues, but some observers have found that the organs from such cases may be infective, and the matter has been the subject of considerable discussion, notably between Ruppert and Schern (1921). In view of the conflicting opinions prevailing the question of the existence and extent of carrier infection must be regarded at present as unsettled.

Infection through the Mucous Membranes.

The nasal discharge from an animal at the height of the disease is very highly infective when applied to the mucous membranes of a healthy animal, and susceptible cattle can be infected in this way with practical certainty by swabbing the mucous membranes of the nose, conjunctivæ, or vagina with the discharge. An interesting fact is that this is not the case with the virus as it exists in virulent blood (Todd and White, 1914), and animals the noses of which are swabbed with highly virulent blood do not become infected unless the swabbing is carried out so freely that an appreciable amount of the blood is swallowed. In the same way swabbing the vagina with virulent blood does not infect. The reason for this difference in the behaviour of the virus as it exists in the nasal discharges and in virulent blood has not yet been elucidated, and explanation is rendered more difficult by the fact that scrapings from the cut surfaces of the liver, spleen and lymphatic glands, unlike virulent blood, infect animals with some ease when applied to the nasal or vaginal mucous membranes.

Virulent blood instilled into the conjunctival sac as a rule causes infection, but here the risk that a certain amount is swallowed is always present, and virulent blood when given by the stomach is generally infective provided that the dose is not too small.

Natural Method of Spread of the Disease.

When cattle plague is introduced into a country previously free from the disease, in the absence of adequate veterinary control its spread is usually rapid and results in an extensive epizootic with high mortality, after which the disease gradually becomes enzootic with irregularly occurring outbreaks in different areas. This state of affairs may persist indefinitely—the disease being mainly carried on by the relatively susceptible young animals which are being continuously added to the population.

The most important part in the spread of the disease is undoubtedly played by actual 'animal to animal' infection through the highly infective discharges from the nose, mouth, gut, &c. As the virus is, however, somewhat rapidly destroyed by desiccation and putrefaction, under ordinary circumstances these discharges rapidly lose their infective character. The sick animal, on the other hand, is a continual source of the infective discharges, and it is to such sick animals that attention must be directed in dealing with the disease. There appears, as yet, no adequate evidence that 'carriers' play any important role in the transmission of the disease.

Immunity.

Active immunity.

The fact that an attack of cattle plague leads to a solid and probably life-long immunity in the recovered animal has led to many attempts to produce such immunity by artificial means. Influenced doubtless by the results of ' variolation ' for small-pox in man, the earlier attempts took the form of ' inoculation ' which was made by means of threads charged with the infective discharge, which were passed through the skin in the form of setons, and it was believed that the mortality amongst cattle contracting the disease in this way was lower than in the case of natural infection. The results of ' inoculation ' were, however, very uncertain; in many herds a high mortality occurred and the method was ultimately abandoned.

Later it was thought that it might be possible to produce a strain of the virus attenuated for cattle by repeated passage through some other species of animal. Experiments on these lines, particularly those carried out by Koch (1897) in Kimberley on sheep and goats, were, however, not encouraging; indeed, repeated passage of the virus through sheep appeared to increase considerably its virulence for cattle.

Numerous attempts have been made to immunize cattle with vaccines prepared by the action of various physical and chemical agents on the virus. The general experience is that the heated virus does not immunize, and Curasson and Delpy (1926) obtained negative results with vaccines prepared by the action of phenol, chinosel and cyanide of mercury on the virus, but were able to immunize with a formalinized virus. The formalin vaccine was prepared by emulsifying the spleen of an animal killed at the height of the fever in 0·2 per cent. solution of commercial formalin in the proportion of 1 gm. of spleen pulp to 5 c.cm. of the solution. After keeping for 40 hours at 35 to 38° C. in the dark, the vaccine was ready for use and a dose representing 12 gm. of the spleen pulp appeared to be sufficient to afford protection. Daubney (1928) also found that formalinized vaccines prepared from spleen pulp confer a high degree of active immunity, and that the kidneys and lymphatic glands could be similarly employed: formalinized blood and liver, on the other hand, appeared to have no antigenic properties.

Kakizaki, Nakanishi and Nakamura (1927) claim to have obtained protection with a vaccine prepared by treating certain organs, notably the thymus and tonsils, of sick animals with glycerin and toluol.

In the course of experiments to devise a method of securing the aggressins of cattle plague, Boynton (1918) tried to extract the virus from the tissues of animals suffering from the disease by grinding the organs with a 0·75 per cent. solution of phenol, and found that these extracts retained their virulence for at least 15 days. He noticed, however, that a number of animals which had received older extracts appeared to be immunized by the primary injection of the extract and suggested that possibly this method might be used in combating the disease. As a result of further work a method of vaccination which is stated to give excellent results in practice has been elaborated (Boynton, 1928). The vaccine used is prepared from the lymph glands, spleen, liver, heart and testicles of a sick animal, these being ground in a special tissue-mill and mixed with glycerin and phenol in the proportion of 900 gm. of tissue to

300 c.cm. of glycerin and 6 c.cm. of phenol. The mixture is heated at 42 to 42·5° C. for 3 hours and then stored in a refrigerator until required for use. Cattle receive a first dose of 2 c.cm. of the vaccine followed after an interval of a week by a second dose of 3 to 7 c.cm., and a week later are said to tolerate the injection of 5 to 10 c.cm. of virulent blood without showing signs of infection.

In 1920 the Government of the Philippine Islands put this vaccine into use on a large scale, and although it is said to have proved of great value in cattle-plague work, it suffered from the disadvantages that after preparation it must be ‘ aged ’ at a low temperature for from 1 to 6 or 7 months, and also that when ready for use it must be used within a comparatively short time as it loses its potency rather rapidly. Attempts to remedy these difficulties resulted in the discovery by Kelser, Youngberg and Topacio (Kelser, 1928) of a vaccine which they found highly efficient. This vaccine is prepared from the lymph glands, spleen and liver of animals killed in the acute stage of cattle plague. The organs are finely ground with an equal weight of physiological saline ; to the resulting emulsion sufficient chloroform is added to give a concentration of 0·75 per cent., and the vaccine placed in cold storage. The chloroform inactivates the virus within several hours, and it is stated that such vaccine can be used immediately after preparation and that it possesses excellent keeping qualities, remaining active for at least one year. Three doses, each of 20 c.cm. of the vaccine given at intervals of a week were found to protect both cattle and carabaos against a subsequent injection of virulent blood. Kelser's vaccine has been further tested by Rodier (1928) who found that, for ordinary purposes under field conditions, a single injection of the vaccine at a dose of 20 c.cm. for carabaos and 10 c.cm. for cattle was sufficient to protect against natural infection. It is to be noted, however, that these results are not in agreement with those of Daubney (1928) who, from experiments carried out in Kenya, concluded that cattle-plague virus is readily and completely destroyed by chloroform and that the virus so killed fails to retain any of its immunizing value.

Immunization with the bile of sick animals. A most interesting discovery was made by Koch (1897), who found that cattle could be actively immunized by the subcutaneous injection of 10 c.cm. of bile taken from a sick animal on the fifth or sixth day of the disease. Ten days after such injections the immunity was pronounced and the animals would support the inoculation of a large dose of virulent blood without harm. The immunity so produced lasted from 3 to 5 months. Not all samples of bile are suitable for this purpose and only dark-green bile should be used ; it must be without odour and should give a white froth on shaking. Samples which are yellow or brown or tinged with blood are unsuitable. When using the method on a large scale it was found advisable to make a mixture of the bile from several animals so as to minimize the risk of using a sample which was inactive. Normal bile has no immunizing action and the bile of animals which have recovered from cattle plague but suffer from or succumb to sequelæ, does not immunize or only does so imperfectly. Koch found that cattle-plague bile is able to render innocuous a considerable amount of virulent blood: in one instance a mixture of 5 c.cm. of the bile with 5 c.cm. of virulent blood did not infect an animal, but produced immunity. He was of the opinion that the addition of virulent blood increased the immunizing power of the bile.

Koch's results were confirmed by Kolle and Turner (1898), and it was found that the blood of cattle which have received a subcutaneous injection of bile is never virulent, so that the virus apparently does not come into the blood-stream. Also when cattle-plague bile is mixed *in vitro* with virulent blood and the mixture injected at once, the animal does not contract the disease, but is immunized, although the bile has had no appreciable time in which to act on the virus outside the body. When cattle-plague bile was allowed to sediment in a tall vessel the supernatant clear fluid showed only a very feeble immunizing action, while the deposit, consisting mainly of mucous and cellular elements, was immunizing. The same result was effected by filtering through a Berkefeld candle. Kolle and Turner state that bile taken from animals during the first three days of the disease has usually no immunizing action and may give rise to cattle plague.

Cattle-plague bile retains its immunizing properties for a considerable time on keeping, Nicolle and Adil Bey (1901) finding it still active after 40 to 85 days. Kolle (1899) centrifuged cattle-plague bile at 3,000 revolutions per minute for half an hour: the supernatant fluid when tested gave only a slight degree of passive immunity, but the deposit after washing and recentrifuging gave rise to a fatal attack of cattle plague, showing that the fully active virus is present in the bile.

A number of attempts to prepare an artificial cattle-plague bile from mixtures of normal bile and virulent blood have been made by Rogers (1900) and others, but so far without success.

The explanation of the production of immunity by cattle-plague bile is difficult, particularly as it has been shown that the virus is somewhat rapidly destroyed *in vitro* by normal bile. The suggestion has been made that the bile may act by setting up an inflammatory action in the tissues and so localizing the virus; but this explanation seems to be ruled out by the fact that normal bile, which sets up an apparently similar reaction to cattle-plague bile, when mixed with the virus does not immunize.

Passive immunity.

The fact that the serum of animals which have recovered from cattle plague when injected into susceptible animals is protective was discovered by Semmer (1893) and Nencki, Sieber and Wyznikiewicz (1898), and was verified by Koch (1897) in his experiments in Kimberley. The protective power of the convalescent serum is not high—some 100 to 200 c.cm. being required to protect an animal—but it was found by a number of observers, Tokishige (1897), Danysz, Bordet and Theiler (1898) and Kolle and Turner (1898), that it can be increased by the subsequent injection of virulent blood.

Preparation of immune serum. A satisfactory method of preparing a powerful immune serum was worked out by Kolle and Turner (1898) who hyperimmunized cattle which had recovered from the disease with a series of about eight injections of virulent blood, beginning with 100 c.cm. and gradually increasing the dose up to a maximum of 4 litres. The protective power of the serum of animals so hyperimmunized was found to be about twenty times as great as that of 'convalescent' serum. The methods employed for the production of the immune serum vary somewhat in different institutes, but are all based on Kolle and Turner's work, though a number of modifications have been introduced, particularly that of Nicolle and Adil Bey, who found that it was not necessary to begin the hyperimmunization with small doses of virulent blood, but that massive doses could be used from the beginning.

The method as used in Egypt (Todd, 1912) was briefly as follows: Clean, susceptible cattle are inoculated with a small quantity of virulent blood, and after a short incubation period they contract a severe attack of the disease. When this is at its height they are bled to death, and the virulent blood so obtained, after the addition of citrate of potash to prevent clotting, is used for the treatment of the animals designed for the production of serum. For this purpose healthy cattle are first immunized by the simultaneous injection of 1 c.cm. of virulent blood on one side of the body and a suitable dose of immune serum on the other side. These injections are followed by a modified attack of the disease, from which, if the dose of serum has been properly chosen, the animal recovers. After complete recovery and when the temperature is normal a massive dose of 4 litres of virulent blood is injected intramuscularly. This is usually followed by a febrile reaction, after the termination of which another dose of 4 litres of virulent blood is given. A fortnight after the last injection, the animal is bled—4 litres being taken from the jugular vein. Three such bleedings, at intervals of a fortnight, are taken, and then another dose of 4 litres of virulent blood given and the process repeated. The blood is allowed to clot and the serum centrifuged, carbolized and bottled. The details of the process of the preparation of the serum are dealt with by Kolle (1909), Todd (1912), and Shilston (1916).

The strength of the serum is tested by determining the amount required to prevent death in a susceptible animal inoculated at the same time with a small dose (0·5 c.cm.) of virulent blood, the injections being given subcutaneously, but on different sides of the body. As the size of the animal influences the amount of serum necessary, this is usually expressed as the number of c.cms. required for an animal of 300 kgm. weight, and the doses are adjusted according to the weights of the animals used.

Apart from a small mortality from a purpuric condition (Rabagliati, 1925) following on the reinforcements, and, in districts where the disease is endemic, from red-water, the serum-producing animals keep in good condition and the strength of their serum increases as the hyperimmunization is continued (Todd, 1912; Hartley, 1914; Rabagliati, 1925[2]).

A detailed investigation of the immune serum by Hartley (1914) showed that the specific protective immune bodies of the serum are similar to, or are associated with, the euglobulins, and are precipitated from the serum in which they occur, either by dialysis or by saturation of the serum with common salt. This behaviour contrasts with that of diphtheria antitoxin in the serum of immunized horses, where the antitoxin is associated with the pseudo-globulin group of the serum-proteins. It is possible that the fact that these two antibodies are prepared in animals of different species may account for the difference, as Pick (1901) found that in the case of goats immunized against diphtheria toxin the antitoxin occurs in the euglobulin fraction.

While in practice, cattle are always used for the production of the immune serum, Nicolle and Adil Bey (1901) were able to prepare quite a good serum in the sheep, but obtained doubtful and probably negative results in the horse and entirely negative results in the goat and in the goose.

The immune bodies in cattle plague immune serum are very susceptible to acids, being entirely destroyed by 0·2 per cent. hydrochloric acid. On the other hand, alkalis in a fairly high concentration (0·4 per cent. sodium carbonate) exert no such destructive action (Hartley, 1914).

Action of the immune serum on the virus in vitro. The immune serum appears to have very little, if any, action on the virus *in vitro*. Kolle and Turner (1898) made a number of experiments with mixtures of the serum and virulent blood, and found that even after 24 hours' contact at room temperature the immune serum did not inactivate more than 1 per cent. of its volume of virulent blood. As the serum used was known to be highly effective when tested by simultaneous injection, Kolle and Turner concluded that the agency of the animal body must in some way be necessary in order to allow it to exercise its specific protective action. Schein (1926) prepared mixtures of immune serum with the centrifuged peritoneal washings from sick animals, testing the mixtures after they had stood for 4 to 6 hours at 28 to 30° C. The results were very variable, but on the whole appeared to be the same as when saline was used in place of the immune serum, and he came to the conclusion that the protective action of the serum was the result of a reinforcement of the natural defensive mechanism of the animal, and was not due to any direct specific action of the serum on the virus. Curasson (1921) made experiments with mixtures of virulent blood and immune serum in varying proportions, from equal parts to 1 in 50, and kept these mixtures at 37° C. At the expiration of two hours the virus was not destroyed in any of the mixtures. After further incubation at 37° C. the relative proportions of the virus and serum appeared to determine the result, as after 2½ hours the virus was destroyed in the 1 to 10 mixture, but not in the 1 to 2. He found that both complement fixation and precipitin tests gave negative results. McKinley (1928) found that the serum of goats which had recovered from cattle plague possessed no 'virucidal' properties for the virus *in vitro*, when virulent blood and the immune serum were mixed in a ratio of 1 to 4 and kept at room temperature for 24 hours or at 37° C. for 6 hours before testing.

Daubney (1928) states that the presence of antibodies in the blood of vaccinated animals can be demonstrated by means of a flocculation test, using as an antigen an alcoholic extract of the freshly pulped tissue of infected spleens with the addition of cholesterol. He found that animals inoculated with virulent blood give a good positive reaction from the seventh day onwards, but that no reaction is given by animals that have been doubly inoculated two years or more previously.

Prophylactic use of immune serum. The protection of cattle by the injection of the serum alone has been used on a very large scale in South Africa, India, Egypt, Russia, Turkey, China and elsewhere; on the whole with good results. The method has the advantage of producing an almost immediate immunity, and there is no risk, as there is in the case of 'simultaneous inoculation', of setting up new centres of the disease or of giving rise to red-water or other parasitic diseases in the injected animals. On the other hand, the passive immunity conferred by the immune serum is of short duration, varying with the natural susceptibility of the animal and with the dose of serum given. Thus Poole and Doyle (1922), in India, found that a single dose of serum gave complete protection for only nine days, and though larger doses gave a longer period of immunity the prolongation was not directly proportional to the amount of serum given. This being the case, if it is necessary to protect the animals for more than a week or two, further injections must be made and the process becomes both laborious and expensive, so that in most countries the use of serum alone has now been replaced by 'simultaneous inoculation'.

Curative action of the serum. The favourable results recorded by earlier workers in the treatment of the actual disease by injections of serum (Kolle and Turner, 1897; Memmo, Martoglio and Adani, 1904, &c.) have not generally been confirmed by later experience, which has as a rule been disappointing; Schern, Mavrides and Major (1920), for example, found that for curative purposes very large doses (500 c.cm.—preferably repeated) must be given, and even then the treatment must be begun at the first appearance of clinical symptoms.

Combined active and passive immunity.

The so-called 'simultaneous method' which was worked out by Kolle and Turner (1898) consists in the simultaneous injection of 1 c.cm. of virulent blood subcutaneously on one side of the body and an appropriate dose of immune serum on the other side: the amount of serum constituting a suitable dose being determined by experiment for each batch of serum. If the dose of serum has been rightly chosen the inoculated animals develop a mild attack of cattle plague, after recovery from which they are solidly immune. In highly susceptible animals the severity of the reaction is determined by the amount of the immune serum given, and it is important that an adequate dose be used, as, apart from the risk of a fatal attack of cattle plague, too violent a reaction may lead to secondary bacterial infections and is very liable to light up any latent protozoal blood infections which may be present.

In districts where cattle plague is enzootic the reactions following on simultaneous injection vary widely in intensity, and a certain percentage of the cattle commonly show no reaction at all; these animals when subsequently tested with virulent blood are found to be immune, but it is, of course, possible that the absence of reaction may be due to a previously existing immunity.

The method of simultaneous injection has now been used on a very large scale in many parts of the world during the past thirty years, and generally speaking has given excellent results. To obtain these it is important that the immune serum used should have been previously tested on local cattle so as to ensure the use of a suitable dose, as the dose required varies within wide limits in the case of different types of cattle; thus in Egypt it was found that, weight for weight, the dose of serum for Cyprus cattle was some six times the amount required for Egyptian animals, and in India the difference in the doses for hill and plains cattle is even greater, hill cattle requiring eighteen times the dose for plains cattle.

The virulent blood used must be microscopically controlled for the absence of blood parasites and must be kept at a low temperature so that its infectivity is not lost. As this is not always easily ensured under practical working conditions, Kolle and Turner (1898) suggested the use of sheep as a source of virulent blood. These were injected with 100 to 200 c.cm. of virulent blood and, although the animals showed no symptoms of cattle plague, their blood, when taken between the third and the eighth day after injection, was virulent and could be used for the purpose of simultaneous inoculation. The injected sheep could be transported for long distances, and the use of their blood avoided the risk of transmitting pyroplasmic and other diseases. The method was used by Kolle and Turner in South Africa and by Memmo, Martoglio and Adani in Erithrea.

After simultaneous inoculation the animals must be kept under good conditions until the temperature reaction is over, as during this period they are very sensitive to cold and exertion.

Carmichael (1928) has recently pointed out that in dealing with outbreaks of cattle plague in localities where immune serum is not available simultaneous inoculation can be carried out on a considerable scale by making use of the serum of animals which have recently recovered from the disease. Over 3,500 head of cattle were inoculated in this way in Uganda with a death-rate of 6 per cent.

The mortality following on simultaneous inoculation in any district is mainly determined by the degree of protozoal blood infection amongst the cattle population. Thus Memmo, Martoglio and Adani amongst some 50,000 animals simultaneously inoculated in the low-lying parts of Erithrea, where protozoal infection is severe, had a mortality of about 3 per cent., while in Upper Egypt during 1923 and 1924 approximately 250,000 cattle were doubly inoculated with a loss of only 0·1 per cent. from all causes following inoculation (Rabagliati, 1925[2]).

Before simultaneous inoculation came into general use it was feared that by creating new centres of infection it would lead to further spread of the disease; this has, however, not proved to be the case.

The immunity following successful simultaneous inoculation lasts for several years, and for practical purposes may be regarded as lifelong, but in view of the encouraging results which have already been obtained with various vaccines, there is every reason to believe that simultaneous inoculation must ultimately give way to the much simpler and less expensive process of vaccination, and investigations directed towards the production of an efficient vaccine would appear to offer the most hopeful path towards a solution of the problem of dealing with the disease.

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CHAPTER XXIV. SWINE FEVER.

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[*Synonyms:* Hog Cholera, Peste porcine, Schweinepest (Virusschweinepest), Peste suina, Peste porcina, Swinedifteritis.]

Description of the Disease.

Swine fever is a febrile, septicæmic infectious disease of pigs, which is accompanied especially by a hæmorrhagic inflammation of the intestinal mucous membrane. In chronic cases there ensues a diphtheroid process in the digestive tract, chiefly in the large intestine.

Clinical Symptoms.

The incubation period after experimental infection by contact is on an average from 6 to 9 days, but more rarely it may be from 10 to 12 days or longer. Dorset (1922), using a potent virus, found that in nearly 200 pigs infected subcutaneously the first symptoms appeared in from 4 to 6 days in 95 per cent. of the animals. The disease begins with an acute febrile reaction, accompanied invariably by an intense conjunctivitis with a muco-purulent discharge. There may be exanthema, nasal discharge, and vomiting followed by diarrhœa and great weakness. In acute cases death occurs in from 6 to 8 days. A secondary pneumonia caused by *B. suisepticus* may complicate the clinical picture. If the animals survive longer ulceration of the intestine may occur.

Post-mortem findings in acute cases are those of a hæmorrhagic septicæmia due to a virus. In chronic cases a secondary necrotic diphtheroid process, sometimes with formation of the so-called ' buttons ' due to *B. suipestifer*, other members of the paratyphoid group or other micro-organisms, is seen. If the lungs are involved there is usually a croupous pneumonia, with or without necrotic foci, accompanied or not by pleurisy and pericarditis. It is now recognized that many lesions formerly considered as diagnostic of swine fever may exist in other infections.

Ætiology.

At first swine fever was believed, as a result of the work of Salmon and Smith (1885), to be due to the pathogenic action of *B. suipestifer.* Later, however, de Schweinitz and Dorset (1903) showed that the disease was transmissible from affected to healthy pigs by the inoculation of filtered bacteria-free blood, thus indicating that the causal agent belongs to the group of ultramicroscopic and filterable viruses. Dorset, Bolton and McBryde (1905), in a publication with an introduction by Salmon, brought further convincing proof in support of the filterable-virus theory and expressed the opinion that *B. suipestifer*, or other bacteria of the coli-typhoid group produce the changes in the intestine secondarily. This opinion was supported by Boxmeyer (1905), Hutyra (1906), Ostertag (1906), and Uhlenhuth, Xylander, Hübener and Bohtz (1908). It has since been amply confirmed and is now generally accepted. In the same way the part played by *B. suisepticus* in producing the secondary pneumonic lesions has been established.

Some workers have produced evidence that there may occur epizootics of swine plague (*B. suisepticus*) and paratyphus of pigs or bacillary hog cholera (*B. suipestifer*) independent of the swine-fever virus.

Modes of Infection.

In natural conditions probably the commonest means of infection is by ingestion. Infection can be produced artificially by the subcutaneous, intracutaneous, intramuscular or intrapulmonary inoculation of virulent material. The disease also results after feeding, by inhalation, or by introduction of a small quantity of virus into the conjunctival sac. The inoculation of extremely small quantities of virus may produce infection ; 0·25, 0·0025, 0·001 c.cm. or even smaller doses of virus result in the development of the disease, especially after the virus has been passaged for some time. The serum separated from infective blood is highly virulent, and there appears to be little or no evidence that the virus is associated with the solid constituents of the blood, although this opinion has been advanced by Dinwiddie (1912) and others.

The transmission of the disease in natural circumstances was the subject of study by the Departmental Committees on Swine Fever (1896, 1897, 1911, 1914, 1915), and their researches would indicate that the disease is conveyed with much difficulty by mediate contagion.

Distribution of the Virus in Organs and Tissues, and Infectivity of Secretions.

The virus can be found in all the organs and tissues and in the blood of affected animals, especially during the early period of infection. All the secretions including the bile are infective to a certain degree, but chiefly the urine.

Evidence of Filterability of the Virus.

As already mentioned, de Schweinitz and Dorset (1903) were the first to show that swine fever could be transmitted by inoculation of bacteria-free fluids. They say ' we have transferred the disease repeatedly from one hog to another by subcutaneous inoculation of certain body fluids, these fluids being always proved by careful bacteriological examination, by filtration through the finest porcelain filters and by inoculation of guinea-pigs and rabbits, to be free from hog-cholera and swine-plague bacilli '. As a result of their investigation and those of others, it is known that the virus will pass through coarse and fine infusorial earth filters such as the Berkefeld and Mandler candles and porcelain filters such as the Chamberland B and F. Kernkamp (1929) obtained an active filtrate after passage of infective fluid through a 2 per cent. alcohol-ether collodion membrane.

Animals Susceptible and Animals Resistant to Infection.

Pigs of all breeds and ages may become infected, but highly bred stock and young pigs are the most susceptible to the disease. According to the observations of Kitt (1916), Bornemann (1923) and Gerlach (1926), wild pigs may also become affected. It is not possible to infect the mouse, rat, guinea-pig, rabbit, dog, cat, horse, donkey, sheep, goat, goose, hen, duck, pigeon, or cattle.

Nature and Properties of the Virus.

The virus is ultravisible. Various investigators have described extremely small bodies in fluids or tissues from infected animals, but these findings have not been confirmed. Uhlenhuth and Böing (1910) and Uhlenhuth (1912) described in smears from the conjunctiva of pigs infected with swine fever, cellular inclusions consisting of very fine granules similar to the so-called trachoma bodies of man. The exact nature of these has not been clearly elucidated. As already stated the virus will pass through filter-candles which hold back the smallest bacteria. It has not been found possible to concentrate it by centrifugalization. There has not been any distinct proof of its multiplication outside the animal body.

Effect of heat. From the observations of various workers it appears that the virus will resist heating at 45 to 46·5° C. for 24 hours and at 58° C. for 2 hours. It is killed after 48 hours' heating at 46° C., 24 hours at 55° C. and 10 hours at 60° C. A temperature of 72° C. for 1 hour or 150° C. for ½ hour is required to inactivate virus in dried blood.

Effect of chilling and freezing. Virulent serum will remain active in the ice-chest at 0° C. or less for from 4 to 6 months. A serum filtrate kept in a freezing mixture was still active after 3 months. Muscle juice from an infected carcass has been found virulent after 30 days, and infection has resulted from the feeding of infective flesh ' chilled ' for 17 days, or frozen for 95 days.

Effect of curing. Birch (1917) showed that ham which had been cured for 6 weeks and then stored for 80 days still contained virus, and Geiger (1927) showed that pickling of pork in 25 per cent. salt did not kill the virus. These facts are of importance with regard to the means of spread of the disease through feeding of offal to pigs.

Effect of desiccation. The virus is very resistant to desiccation. Blood or serum dried for 15 days may still produce the disease, and recently, Miessner and Geiger (1926) showed that blood and serum carefully dried by hot air in a Faust-Heimschen apparatus was still virulent after 3 weeks.

Effect of sunlight and ultraviolet light. Virus exposed to direct sunlight for from 5 to 9 hours has been found virulent, but ½ to 1 hour's irradiation with ultraviolet light from a ' Sunlamp ' kills it.

Effect of putrefaction. The virus is relatively sensitive to putrefaction. Buried viscera which have putrified become non-infective in 8 days. Virulent urine mixed with putrifying fæces becomes inactive in 24 hours at 22° C.

Action of disinfectants and glycerin. The virus is fairly resistant to most ordinary disinfectants. In serum filtrate it will resist 1 per cent. antiformin for 24 hours or 2·5 per cent. for 1 hour, but the latter concentration will kill it in 2 hours; 2·5 per cent. formalin will render virulent serum non-infective after 15 days in the ice-chest; the same concentration will not inactivate it in 1 hour at room temperature; 5 per cent. formalin kills it in 2 hours. Eight days' contact with 1 per cent. corrosive sublimate will not inactivate the virus. The virus is resistant to glycerin. After 1 month in 33 per cent. it is still fully virulent. Virus which has been in contact with 3 per cent. phenol for 8 days, 2·5 per cent. phenol for 3 days and 1 per cent. phenol for 4 days is still quite active. In defibrinated blood and 0·75 per cent. phenol at 5° C. it has been found virulent after 162 days and in blood with 0·5 per cent. phenol and 1 per cent. glycerin at 17° C. after 3 years. Three per cent. cresol water and 3 per cent. soda lye will inactivate the virus in 1 hour, and 5 per cent. bleaching powder in ¼ hour. The two former have been most commonly used for disinfection.

Immunity.

Natural immunity to swine fever has never been observed. The greater resistance to infection exhibited by older animals is believed to be due to their having had a mild attack when young. Animals recovering from swine fever are generally immune for their entire lives. In general, young pigs of immune sows have a fairly high degree of immunity to infection, which may be such that they will resist an inoculation of as much as 5 c.cm. of virulent blood, but it will apparently not always protect them against natural infection in severe epizootics. The immunity disappears some few weeks after the cessation of suckling, and is believed to be conferred by antibodies in the milk.

Active immunity. De Schweinitz, Dorset (1903) and Boxmeyer (1905) were the first to establish from experimental observations that recovery from the natural or artificial infection left immunity to artificial or natural infection. This has been abundantly confirmed, and has led many investigators to try to produce an active immunity by inoculation of virus, killed or attenuated by the various physical or chemical means which have been employed in other diseases. However, it may be stated that none of the methods employed have been satisfactory in producing an absolute active immunity. It is believed that some attempt is now being made to produce a formolized vaccine similar to those which have been used in foot-and-mouth disease, distemper, rinderpest, and yellow fever, but no published results have yet appeared.

Immunity with immune serum alone. Preisz (1898) was the first to conceive the idea of employing the serum of pigs recovered from swine fever for immunizing purposes. The serum from a pig taken three weeks after a severe attack of swine fever was inoculated in doses of 10 c.cm. to pigs, which were subsequently exposed along with controls to natural infection. The inoculation of the serum proved to have some protective action. It has now been established by the investigations of Dorset, McBryde, Niles and Rietz (1919), Hutyra and Kõves (1916), Hutyra (1920), Uhlenhuth and Miessner (1926), Uhlenhuth, Miessner, Geiger and Baars (1925), Donatien and Lestoquard (1929) and others that the hyperimmunization of pigs that have acquired an immunity from a natural or acquired infection with swine-fever virus produces a serum which will protect susceptible animals against artificial or natural infection for some 3 to 4 weeks. Apparently, also, such serum possesses some curative and protective action on pigs in the incubation stage. In some accurately controlled observations in Germany, Uhlenhuth and his collaborators tested his serum on over 300 animals in severely infected herds. Of this number 61, or about 18 per cent., died of swine fever, while out of an almost equal number of controls over 50 per cent. succumbed. As good, and even better results in some instances, have been reported by American workers. In one test on over 40 herds, comprising over 2,000 animals, the subsequent losses from swine fever were only about 3 per cent. Hutyra and Kõves in Hungary inoculated with serum over 100,000 pigs in some 800 infected herds with a percentage loss from swine fever of 9·4.

In the preparation of the hyperimmune serum the importance of using highly virulent material for administration is recognized as all-important. The testing of the serum for its potency and the determination of the dose to be administered have also to be carefully controlled. It is evident that all sera which are prepared are not of equal potency, and in many cases unless accurately tested they may be quite useless.

Concentrated immune sera have been prepared by similar methods to those used for tetanus and diphtheria antisera.

Immunity with immune serum and virulent blood. It was found that in infected herds, pigs which had been passively protected by serum alone frequently proved solidly immune. Later it was believed that such animals had acquired an active immunity. This led to the employment of a ' simultaneous method ' of immunization which was originally used by Dorset and his collaborators. Immune serum is given along with a dose of fully virulent blood. The active immunity so given is very lasting.

This method of immunization, as also that of the ' serum alone ' method, has its special indications, as apparently it is believed that there is some danger of the pigs inoculated by the ' simultaneous method ' being a source of infection. The ' simultaneous method ' has been used with much success by many, but frequent mention has been made in the literature, to ' breakdowns ' which may occur in immunizing experiments in the field. Such breakdowns may be due to many factors including the use of insufficient doses of virus and the vaccination of pigs while yet too young. In some cases the ' break ' is apparently not due to swine-fever virus, but to the fact that *B. suisepticus*, latent in the animal, becomes active as a result of the lowered resistance produced by the administration of the hog-cholera virus in the simultaneous method. In view of the observation of Montgomery (1921) and Steyn (1928), who described epizootics among pigs in Africa due to a filterable virus, one might also be led to consider whether, in swine fever as in foot-and-mouth disease, there do not occur different types or strains of virus. Montgomery (1921) found that pigs immune to a strain of swine-fever virus obtained from England contracted a fatal infection when inoculated with the East African virus.

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CHAPTER XXV. VIRUS DISEASES OF RABBITS AND GUINEA-PIGS.

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The virus diseases of rabbits and guinea-pigs are of interest for three main reasons. Firstly, certain of them may cause epizootics amongst laboratory stocks and be a cause of high mortality. Secondly, they may lead to the drawing of erroneous conclusions by workers with viruses who are ignorant of their existence and manifestations. Virus III, for instance, when encountered by workers with chicken-pox, was found to produce intranuclear inclusions just like those of varicella; others meeting it while studying rheumatic fever, found that it produced a fibrinous pericarditis; while another, seeking for the cause of scarlet fever, noted a scarlatiniform eruption in rabbits inoculated with the virus. Finally, these viruses may throw considerable light on those which affect man and other animals. Particularly suggestive is the finding that the salivary virus, to which the majority of adult guinea-pigs are apparently immune, is yet harboured within the cells of the salivary ducts of such guinea-pigs, apparently throughout their lives. Though the evidence is less complete, it seems likely that Virus III is similarly carried by immune rabbits. The application of these ideas to the study of immunity to virus diseases in general opens up a wide field. The diseases considered in this chapter appear to represent well-defined entities, but it must be remembered that they have only been described by few, widely-separated workers, and that few cross-immunity experiments have been carried out between the different viruses considered here and between them and other infections transmissible to laboratory animals.

Infectious Myxomatosis of Rabbits.

The name of this disease gives a key to its most interesting feature: it possesses at once some of the characters both of an acute infection and of a new growth. Several workers have suggested analogies between the causative agent and that of the Rous chicken sarcoma. Epizootics of the disease have been recorded amongst laboratory rabbits in Uruguay, Brazil and Argentina at irregular intervals since it was first described by Sanarelli (1898).

Symptomatology. Severe bilateral blepharo-conjunctivitis is the first symptom. Small subcutaneous tumours soon appear scattered over the body, especially on the ears. The mouth, the anal and genito-urinary orifices become inflamed, and thickening of the mouth and eyelids produces a leonine facies. Wasting and fever occur and death follows, usually within 4 or 5 days of the onset. Rabbits in contact with infected animals or inoculated by any route develop symptoms after an incubation period of 4 or 5 days (Sanarelli, 1898) or rather longer (Splendore, 1909). Virulence can be increased by passage, and death may then occur in 4 or 5 days after inoculation, the rabbits appearing to die of septicæmia, and showing no symptoms or only conjunctival congestion (Aragão, 1927). Moses (1911) also found that the myxomatous tumours might be absent; he found thickening at the base of the ears the most constant symptom. The white blood-count rises during the course of the disease (Hobbs, 1928). Cage infection readily occurs and epizootics may kill 100 per cent. of a stock (Aragão, 1927) or may die out spontaneously (Splendore, 1909).

Pathological anatomy. Sanarelli (1898) records that death leaves the whole rabbit distorted and disfigured. Gelatinous vascular tumours are scattered through the subcutaneous tissues; they contain a serous fluid (Aragão, 1927). The spleen and lymph-glands are generally enlarged and the liver engorged; orchitis also is found.

Histology. There appears to be a specific proliferative and degenerative process attacking the connective tissues of the tumour-like masses, of the spleen and lymph-glands, and of the thickenings of the eyelids and muco-cutaneous junctions. Star-shaped cells predominate in the myxoma-like tissue, which, according to Lipschütz (1927) is derived from a mucoid degeneration of the collagen. Aragão (1927) alone attributes all the swelling to œdema. In the lymph-glands the changes appear to begin in the cells lining the lymph-vessels; finally, the lymphocytes are wholly replaced by myxomatous cells (Rivers, 1928). Especially in the larger swellings much capillary proliferation and accumulation of polymorphonuclear leucocytes and other cells are seen. The appearances thus suggest an acute inflammation rather than true tumour-formation.

Almost every worker with the disease has described 'specific bodies' which he believes to be different from the 'specific bodies' found by others: there are 'trachoma-like bodies' (Splendore, 1909), 'small round bodies' (Moses, 1911), intranuclear 'chlamydozoa' or 'strongyloplasmata' (Aragão, 1911, 1927), and granules in the cytoplasm believed by Lipschütz (1927) to represent a new class of organism called by him 'Sanarellia'. It is at any rate clear that profound degenerative changes occur in the connective tissue-cells. Quite different are the changes in the epithelium overlying tumour masses described by Rivers (1927) and figured by Rivers (1928^1) and by Hobbs (1928). Within the cytoplasm of enlarged epithelial cells acidophilic bodies appear which increase till they may involve all the cytoplasm; the cells finally dissolve, leaving distinct vesicles. Rivers (1927) emphasizes the different way in which the disease attacks the epithelial and connective tissues.

Animals susceptible. Laboratory rabbits have been uniformly susceptible to infection, whereas wild South American rabbits (*Lepus* *brasiliensis*) could only exceptionally be infected (Moses, 1911). Monkeys, cats, dogs, goats, guinea-pigs, rats, mice, fowls and pigeons have proved refractory.

Therapeusis. Intravenous neosalvarsan and tartar-emetic failed to influence the course of the disease (Hobbs, 1928).

Ætiology. Infectious myxomatosis has been accepted as a virus-disease on the grounds that no organism is generally recognized to be visible in the lesions, that no organism has been cultivated from them, and that the infectious agent is transmissible in series, filterable and resistant to glycerin.

Filterability. Neither Splendore nor Moses could filter virus contained in suspensions of infective tissues through Chamberland candles, but Moses (1911) found that it would pass Berkefeld 11 to 14 filters. Hobbs (1928) found that it would filter through Berkefeld V filters which were impermeable to *B. prodigiosus* ; he used a negative pressure of 20 cm. of mercury. The virus would not pass Berkefeld N or Chamberland L5 and L7 candles. Some light is thrown on the difficulties of filtering the virus by the fact that it can be adsorbed by powdered Berkefeld and Chamberland candles. Hobbs also studied its adsorption by a number of other substances.

Properties of the virus. Virus myxomatosum survives for months at 8 to 10° C., but soon loses its activity at room temperature. It is readily killed by heat : 50° C. for 15 minutes (Hobbs) ; 50° C. for an hour or 60° for 30 minutes (Moses). It withstands 50 per cent. glycerin for a year (Hobbs) and is killed by 0·5 per cent. carbolic or 0·2 per cent. corrosive sublimate in 4 days in the cold (Moses). Sanarelli (1898) and Moses (1911) record a number of other experiments on the effect of antiseptics. It will survive in a range of pH from 4 to 12 for 15 minutes, but is inactivated on either side of those limits. It can also be inactivated by ultraviolet radiation (Hobbs, 1928).

Transmission experiments. The virus is equally infective by whatever route it is inoculated ; it is present in the blood, even during the incubation period, and in all organs ; 0·025 c.cm. of serum (Moses, 1911) or even 0·001 c.cm. (Aragão, 1927) suffice to infect ; Hobbs (1928) found a tissue emulsion infective in a 1 : 100,000 dilution. A tumour usually appears locally at the site of inoculation and tumours will especially localize where hair has been plucked out when the virus is injected intravenously.

Cultivation. Though no specific organism has yet been cultivated by any worker, a Pasteurella commonly appears as a secondary invader (Aragão, 1927). During attempts at cultivation, Hobbs (1928) found that the virus still survived for a week at 37° C. in Locke's solution and certain media, including tissue cultures ; but no definite multiplication was shown.

Immunity. Rabbits have not as yet been successfully immunized by virus which has been inactivated in any way (heat, dilution, antiseptics) (Sanarelli, 1898 ; Moses, 1911). No protective, curative or 'viricidal' properties have been detected in the serum of inoculated animals, even in that of two of Sanarelli's rabbits which survived infection and were hyperimmunized with virulent blood.

Virus III Infection of Rabbits.

Rivers and Tillett (1923) in New York, in their search for the virus of chicken-pox, inoculated material from patients into the testes of rabbits. Though no reaction occurred they carried on successive transfers at 3-, 4- or 5-day intervals from rabbit's testis to rabbit's testis. After the third passage they found that they were transferring an agent which produced in rabbits fever and acute orchitis. The characters of the agent led them to believe it to be a virus and they referred to it as Virus III, having worked chiefly with the third strain encountered. Other workers, using a similar technique, have met with the virus: Miller, Andrewes and Swift (1924) working on rheumatic fever in New York, M'Cartney (1924–5) studying scarlet fever in England, and Doerr (1926) in Switzerland. Andrewes and Miller (1924) brought the virus to light in a series of rabbits in which they started with inoculations of apparently normal rabbit-blood. Using the technique described, workers have never found evidence of the presence of the virus until after the third or fourth passage. It seems likely that Virus III is a natural parasite of domestic rabbits, but the natural course of infection in the rabbit is unknown; it has so far only been demonstrated under the artificial conditions described.

Symptoms in inoculated animals. Rabbits inoculated intratesticularly with suspensions of infected testes develop swelling of the testis in about 3 days. After a further 1 or 2 days the reaction is at its height and the rabbits commonly have fever (104 to 106° F.). The animals recover, apart from possible testicular atrophy; inflammation does not spread to the opposite testis. Intradermal inoculation produces lesions which are red, slightly raised, and up to 30 or 40 mm. in diameter; they appear usually on the third or fourth day. Testicular suspensions diluted 1 in 10 or even 1 in 1,000 (Rivers and Tillett, 1924[1]) will still react. Virus rubbed into scarifications on the skin produces lesions less regularly. Intracerebral inoculation is usually followed by fever, but no other definite symptom: at times a fatal encephalitis occurs (Rivers and Stewart, 1928). Some reaction is also produced on the scarified cornea.

There is some evidence of cage infection, the criterion of a natural infection being the spontaneous development of immunity (Rivers and Tillett, 1924[1]). Due regard was paid to the occurrence of a certain percentage of naturally immune rabbits (vide *infra*).

Pathological anatomy and histology. Severely infected testes become swollen, firm and hæmorrhagic, and the scrotum, œdematous. Sections show that the germinal epithelium is disorganized, the interstitial tissues are filled with an exudate containing lymphocytes, macrophages, plasma-cells and polymorphs in varying proportions; mononuclear cells usually predominate. In testes examined 3 to 5 days after inoculation and stained by eosin and methylene blue, hæmatoxylin and eosin, Giemsa or Mann, a large number of cells in the interstitial tissues, apparently macrophages, contain intranuclear inclusion bodies, very much like those described in the lesions of herpes febrilis or of varicella (oxychromatic degeneration of Luger and Lauda). A large pink acidophilic body is seen to lie in the middle of the nucleus, the basophilic chromatin of which is condensed into a narrow, dark-blue ring separated by a clear zone from the central mass (Rivers and Tillett, 1924[2]). Later than five days from infection, these inclusions are hard to find. After a week or so the testes may begin to atrophy and the interstitial tissues will be found packed with lymphocytes. In less severe infections lesions similar to those described are found focally in the testes.

In any other part of the body inoculated, skin, fat, voluntary muscle, myocardium, pericardium, eye, or brain, there occurs local inflammation with mononuclear cells preponderating, and the intranuclear inclusions can be found. In the cornea and ciliary body, these inclusions occur in epithelial cells and Rivers and Stewart found them in nerve-cells (1928); elsewhere, they have only been seen in interstitial tissues in cells believed to be of mesodermal origin. If tissue cultures of rabbit's testis are infected with Virus III, the inclusions will form *in vitro* (Andrewes, 1929).

Reasons for believing that the agent is a virus. The active agent has been propagated in series through several hundred rabbits since 1923. No organism has hitherto been cultivated from the lesions, nor seen in smears or sections. The virus passes a Berkefeld filter and resists glycerin. The immunity reactions are comparable with those of other viruses. The intranuclear inclusions are hardly distinguishable from those occurring in certain other virus diseases.

Filterability. Rivers and Tillett (1924[1]) succeeded in filtering the virus through Berkefeld V and W filters which were impermeable to *B. influenzæ.* It does not, however, filter readily: a virus, active in the skin at a dilution of 1 : 1,000 was filtered, and the filtrate only produced a lesion when undiluted.

Physical properties of the virus. Virus III is inactivated by heating to 55° C. for 30 minutes. It will survive for six days in the cold in Ringer's solution. A Berkefeld filtrate was preserved in Locke's solution for 17 days in the cold; in the presence of 40 per cent. glycerin survival was for 24 days. Material frozen and then dried survived for 16 months (Rivers and Stewart, 1928), while an infected testis frozen solid by the writer and kept at −5 to −10° C. is still infective after 231 days.

Transmission experiments. Inoculated rabbit testes remain infective for five days; after this time virus can only be recovered from them scantily and irregularly; testes are free from demonstrable virus in a month and probably much earlier. It is therefore of great interest that the virus can persist in a transplantable neoplasm for at least 54 days, and probably throughout the life of the tumour-bearing rabbit (Rivers and Pearce, 1925); the reason for this is obscure. Attempts to transfer Virus III to guinea-pigs, mice and rhesus monkeys have been unsuccessful.

Active immunity. Rabbits inoculated with living virus by almost any route develop an immunity, as shown by the absence of reaction to an intradermal inoculation 5 days to 6 months later (Rivers and Tillett, 1924[1]). Heat-inactivated virus does not immunize. Virus inoculated into the testes of immune animals could no longer be recovered two hours later. Rabbits partially immunized by inoculations of very small doses of virus were found (Andrewes, 1928[1]) when tested three weeks later to respond to intratesticular inoculations by an allergic reaction. This was characterized histologically by an intense lymphocytic reaction from which the typical inclusions were absent. There is no cross-immunity between Virus III and the viruses of herpes or vaccinia.

Passive immunity. The serum of immune animals contains ' viricidal ' antibodies. Active virus mixed with such serum and inoculated in the skin with or without previous incubation produces no reaction. Serum inoculated intradermally 24 hours before the virus confers a local passive immunity on that area of skin (Rivers and Tillett, 1925), but passive immunization of the whole rabbit has not been achieved. As with all ' viricidal ' sera, the mode of action is obscure, but there is some evidence that it does not directly kill the virus *in vitro* (Andrewes, 1928[2]).

Distribution of the virus. The particular interest of Virus III centres round the mechanism by which it has been brought to light from apparently normal rabbits. It seems most likely that it is carried in small quantities, possibly in the testes, by some rabbits, perhaps those which have passed through a natural infection, and that a few passages through non-immunes increase it to a point at which its effects are obvious. It has also been suggested that it is a non-living transmissible ferment acting on cells. In favour of the first view are these facts: in New York, Rivers and Tillett (1923) and Andrewes and Miller (1924), readily brought to light fresh strains of virus by a few testis to testis passages; Rivers and Tillett (1924[1]) also found that 15 to 20 per cent. of stock rabbits were naturally immune and had antibodies in their sera. In London, Andrewes (1928[1]) failed to discover a fresh strain of virus and correspondingly found no evidence of natural immunity among his rabbits. It is thus suggested that Virus III is endemic only amongst certain stocks of rabbits. Its existence must, however, be borne in mind by all who are using rabbits for experimental work involving serial passages.

Salivary Virus of Guinea-Pigs.

Cole and Kuttner (1926), in New York, found in the ducts of the submaxillary salivary glands of 84 per cent. of apparently normal adult guinea-pigs enormously swollen epithelial cells; these cells varied from twice the normal size up to 40μ in diameter. They usually occurred in groups and were surrounded by areas of mononuclear cell-infiltration. The ducts of the serous part of the salivary gland were chiefly affected, less commonly the mucous part of the gland; at times the abnormal cells could be found in the so-called parotid gland. When stained by methylene blue and eosin, the cytoplasm of the swollen cells took on the blue stain more deeply than usual and the nuclei were seen to contain large acidophilic masses. These, though larger and more deeply staining, were essentially of the same structure as the intranuclear inclusions of herpes simplex, being separated by a clear halo from the somewhat irregular, strongly basophilic, thickened nuclear membrane. Similar cells had previously been seen by Jackson (1920), who believed them to represent a protozoan parasite. They have recently been found by the writer amongst London guinea-pigs. Cole and Kuttner failed to find similar structures in the salivary glands of the rabbit, rat, mouse, dog or cat.

Transmission experiments. In guinea-pigs less than a month old, these inclusions were rarely present. When, however, such young guinea-pigs were inoculated into the salivary glands with emulsions of the salivary glands of old guinea-pigs and were killed 2 to 12 days later, areas of cellular reaction had appeared in the glands, and inclusions could usually be found in the ducts, though they were contained in cells which were much smaller than those present in spontaneously affected adult animals. Similar injections into the testis, lung and tongue also produced a local mononuclear-cell reaction and caused the appearance of intranuclear inclusions; in the testis these occurred both in the cells of the interstitial tissues and of the tubules. Intracerebral inoculation caused an acute meningitis accompanied by fever and leading to convulsions, weakness and death in about five days. The meningitic exudate consisted of mononuclear cells, many of which, though of normal size, contained inclusions; the brain itself was scarcely affected.

Unexpected difficulties were met with in effecting serial passages from brain to brain or by other means. Later, Kuttner (1927) effected seven serial passages from submaxillary gland to submaxillary gland. She also found that in young guinea-pigs inoculated subcutaneously, intravenously or intraperitoneally with the salivary glands of old guinea-pigs, inclusions could be found in the submaxillary glands 9 to 15 days later. By successive transfers from salivary gland to subcutaneous tissues in this manner, seven serial passages were again achieved. In guinea-pigs from which the submaxillary glands had been removed by operation, subcutaneously introduced virus localized in the parotids.

Rabbits, rats, kittens, dogs, mice, a rhesus monkey, chickens and pigeons proved refractory to intracerebral inoculation of the virus.

Properties of the virus. The active agent was believed to be a virus on account of the following properties: (1) It was transmissible in series; (2) no organism was cultivated from lesions aerobically nor anaerobically; (3) it was inactivated by heating to 54° C. for 1 hour; (4) salivary glands containing it when placed in 50 per cent. glycerin retained their activity for 10, though not for 28 days; (5) it passed a new Berkefeld N candle which was impermeable to *B. coli.*

Immunity. Adult guinea-pigs whose salivary glands contained the virus were resistant to intracerebral inoculation. Young guinea-pigs which had received living virus by various routes 12 to 15 days previously were also refractory; heat-killed virus, on the other hand, conferred no immunity. No ' viricidal ' properties could be demonstrated in the sera of immune animals when mixed with small quantities of active virus, and, after standing 30 minutes at room temperature, inoculated intracerebrally into young guinea-pigs. Unpublished observations of the writer's indicate, however, that with quantitative experiments it is possible to demonstrate the presence of antibodies in such sera.

In all the work above described, Cole and Kuttner carried out careful controls to eliminate error arising from the endemic occurrence of the virus amongst their guinea-pigs. The virus is of great theoretical interest from two points of view. First, no explanation is forthcoming at present of the facts that when introduced intracerebrally it will regularly kill and yet serial passages from brain to brain are unsuccessful. Secondly, it provides an example of lasting immunity to a virus associated with a persistent, latent infection with that virus.

Guinea-Pig Paralysis.

Römer (1911[1 & 2]) described a paralysis occurring sporadically among guinea-pigs in Marburg. The disease has not been recorded from elsewhere.

Symptomatology. Affected animals showed fever and loss of weight followed by increasing hypotonia, usually of the hind-limbs, but occasionally of the fore-limbs. Bladder symptoms were common. Paralysis of the hind-limbs was finally complete, and the animals invariably died after an illness running a course of 3 days to 4 weeks, most frequently 8 to 10 days.

Pathological anatomy. The only noteworthy lesions were in the central nervous system, where, in the late stages, there was a diffuse meningo-myelo-encephalitis. The meninges, which were affected most, showed an exudate, predominantly lymphocytic, which extended inwards along the vessel sheaths into the grey matter of the brain and cord. In the cord, the lumbar region was chiefly involved. Destruction of nerve-cells was late and perhaps secondary to the other changes; neuronophagia was rare.

Properties of the virus. No bacteria could be cultivated or demonstrated in infected organs. The active agent readily passed Berkefeld filters which did not allow *B. prodigiosus* to pass. It withstood 50 per cent. glycerin for 10, but not for 36 days. In affected animals it was present in the brain, spinal cord and abdominal lymph-glands, occasionally in the spleen and liver, but not in other organs. Transmission to other guinea-pigs was carried on for five passages by intracerebral inoculation; other routes were unsuccessful. There was an incubation period of 9 to 23 days. No attempts were made to convey the disease to animals other than guinea-pigs.

Guinea-Pig Epizootics attributed to Virus Infection.

Epizootics amongst guinea-pigs attributable to virus-infection have been described as occurring at the Lister Institute in London by Petrie and O'Brien (1910) and in Turin by de Gasperi and Sangiorgi (1913). The descriptions suggest that the diseases dealt with are identical, though this is not certain.

Symptomatology. Emaciation, diarrhœa, roughening of the fur and finally weakness and convulsions led on to death; this occurred in 2 to 17 days after inoculation or exposure to infection. Guinea-pigs placed in cages previously occupied by infected animals succumbed themselves.

Morbid anatomy. Nothing striking was seen; only congestion of the viscera and dilatation of the intestines. No characteristic bodies could be found histologically.

Ætiology. The Lister Institute epizootic was associated with a Salmonella infection. Petrie and O'Brien believed that their filtration experiments and the epidemiological evidence proved that the Salmonella was only a secondary invader: guinea-pigs fed with cultures of the Salmonella were not readily infected and contacts with such animals rarely died, yet the epizootic mortality was 90 per cent. The heart's blood of infected animals, though sterile on culture, would transmit the disease in a very small dose.

Filterability. Both sets of workers found that the virus in infective blood or organ-suspensions would pass a Berkefeld filter. De Gasperi and Sangiorgi record that their filters held back *B. prodigiosus* and that their filtration was at 3 atmospheres' pressure for 10 to 15 minutes.

Properties of the virus. The virus survived for 15, but not 30 days at 1 to 6° C. and could be kept dried for 10 days. It survived heating for 1 hour at 65 to 68°, but no details as to the technique of this experiment are given (de Gasperi and Sangiorgi). Infective brain placed in pure glycerin survived 8, but not 23 days. A filtrate mixed with an equal volume of 5 per cent. carbolic was still alive after half an hour. Petrie and O'Brien found broth-cultures still infective after aerobic incubation at 37° C. for 14 days. The virus could be carried down with a globulin precipitate (Sangiorgi, 1914).

Transmission experiments. De Gasperi and Sangiorgi could infect with virus in a dilution of one in a hundred million; one would like to be assured that a fresh pipette was used for each dilution. Inoculation by any route would infect guinea-pigs. All organs were infective; so were filtrates of the stools, urine and bile. Mice were insusceptible. So were rabbits, except after intravenous inoculation; two so inoculated died after 10 and 23 days and their brains contained active virus.

No immunological studies are reported.

The experiments as they stand strongly suggest a virus as the cause of the epizootic. Yet where such an infectious disease is concerned unusual care is necessary in interpreting any experimental result. Only the occurrence of fresh epizootics and repetition of the experiments can establish 'Meerschweinchenpest', as de Gasperi and Sangiorgi term it, as a virus-disease.

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CHAPTER XXVI. AFRICAN VIRUS DIASES OF SHEEP.

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Blue Tongue.

Synonyms: Malarial Catarrhal Fever, Blauw Tong, Besiekte, Vuel Bek.

Definition.

Catarrhal fever of sheep is a widespread disease in South Africa. It is characterized by inflammation of the mucous membrane of the mouth and tongue, the upper respiratory passages, intestines and corium of the hoofs. It is caused by an ultravisible and filterable virus which is probably transmitted by insects. The duration of the disease is about three weeks. In certain years and in certain flocks very few animals die, in other cases 30 to 50 per cent. of the sheep succumb. Usually the sheep die from general weakness and emaciation. The disease is found in low-lying territories and is more prevalent in the months of March, April and May, i.e. during the wet season.

Ætiology.

The former assumption that Blue Tongue was produced by an intracorpuscular parasite has not been substantiated. The causal agent is an ultravisible virus which is found in the blood and organs of diseased sheep, and can pass through a Berkefeld filter. The blood is virulent in very small doses. The serum is likewise virulent and even in the same degree as the whole blood (Theiler, 1906). The virus is easily preserved. In Edington's mixture (1,000 parts blood, 1,000 parts glycerin, 1,000 parts water and 1 part carbolic acid) the blood is still virulent after 2 years. The virus is not destroyed by putrefaction (Theiler, 1906), and drying of the blood does not always remove its infectiousness, although the virus is attenuated to some extent. The method of natural transmission of the disease has not been elucidated. It is not directly contagious ; no passage of the disease has been effected by placing healthy with diseased sheep. Possibly blood-sucking flies convey the infective agent. It is significant that the malady can be prevented by removing sheep out of the marshy area, and by keeping the flock under shelter from before sundown till after sunrise. Theiler (1905) made the observation that in regions where catarrhal fever exists, *Culex* is prevalent, whereas *Anopheles* and *Stegomyia* are more common where horse-sickness prevails.

The disease can be transmitted artificially by inoculation of the blood or serum of sick animals, subcutaneously, intravenously, intratracheally, or intraperitoneally, to fresh sheep. It has never been produced by administration of virulent material with the food.

Although catarrhal fever of sheep and horse-sickness are very similar in nature, there does not appear to be any proof that they are identical. The virus of horse-sickness is not capable of being passed to sheep, nor the virus of catarrhal fever of sheep to horses.

Animals Susceptible and Animals Resistant to Infection.

The susceptibility of sheep varies with the breed. Merino sheep are supposed to be the more susceptible. Fat-tailed sheep and Persian sheep are somewhat resistant. Imported stock are more susceptible than those in an infected area. Young animals from 1 to 2 years are more severely affected than old sheep. Dixon (1909) observed that sucklings do not become affected.

Most investigators have failed to transmit the disease to other animals, such as horses, cattle, dogs, goats and rabbits. Spreull (1905) inoculated virulent blood to goats and calves, and although these animals did not become ill, their blood remained infective for sheep for some time. Under natural conditions, catarrhal fever does not appear to affect these animals.

Immunity.

Sheep which have survived an attack of the disease possess a certain degree of immunity, but this is by no means complete. Indeed, experience has taught that such animals may again be affected the following year, but as a rule the percentage which succumb to the second attack is exceedingly small. Immunity thus acquired may be broken down by the inoculation of a large quantity of virulent blood ; in some cases even death may result. In favourable cases the repeated inoculation of virulent blood produces a fairly strong immunity to natural infection.

Sheep which have passed through the disease are not virus carriers. The virus usually disappears from the blood soon after the fall of the fever, but it has been found there up to the thirtieth day after the end of the febrile reaction, and in one recorded case the blood taken on the fiftieth day proved to be infective, but this must be taken as an exception.

In early experiments, Spreull (1902) attempted, but without success, to modify the virus by the action of various chemicals, including formalin and carbolic acid, with a view to preparing a vaccine. Bile from recovered sheep was shown to have no protective action. Later, he showed that the serum of a hyperimmune animal possesses a high protective property, and in 1905 he recommended the use of 4 c.cm. of hyperimmune serum and 2 c.cm. of virus to be given simultaneously as a protective inoculation. Theiler (1906) employed a similar method. He found that the serum of sheep which had been hyperimmunized by injection of 500 c.cm. of virulent blood had a curative and protective action. The administration of 10 c.cm. of such serum sometimes arrests the course of the disease, and in this way the spread may be limited, but the passive immunity so produced does not last long. He found also that no active immunity resulted after the inoculation of a virus-serum mixture (5 c.cm. of serum and 2 c.cm. of virus) nor did such inoculation produce any reaction.

Theiler (1908) found that the virus becomes attenuated by passage. Of 319 animals inoculated with virus after the eleventh to eighteenth passage none died, while with virus from the first to the tenth passage the mortality was 11 per cent. This vaccination method has since been tested on a large scale, and the results have been satisfactory. In the first year the mortality among the uninoculated sheep was 11 per cent., while among the vaccinated sheep it was 0·6 per cent. The inoculation produces a slight febrile reaction and the immunity produced lasts at least one year. Abortion has in some cases followed upon the inoculation, but it can be prevented by not putting the rams with the ewes until one month after the inoculation.

Dixon (1909) reported the good results obtained with the vaccine of Watkin-Pitchford, which is a virus attenuated by heating to 44 to 47° C., but this method does not seem to have been tried in the field.

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Nairobi Disease of Sheep.

Definition.

A specific febrile disease of sheep and goats characterized by an acute hæmorrhagic gastro-enteritis. The cause of the disease is an ultravisible and filterable virus which can be conveyed by the 'brown' tick (*Rhipicephalus appendiculatus*).

Epizootiology.

The disease was first reported in Nairobi by Edwards (1910). Montgomery (1917) stated that it had been found to exist in the Kikuyu country, which extends from Mount Kenya to Nairobi, on several occasions between 1910 and 1915. The mortality varies from over 30 per cent. among grade and merino sheep to 70 per cent. among native sheep.

Ætiology.

The disease is produced by a virus which is present in the circulating blood and which is capable of passing through Berkefeld and Chamberland filters. The blood is always virulent during the temperature reaction, and may remain so for about a day after the return to normal. Serum from clotted blood taken during the infective period is virulent. The disease has been transmitted by inoculation of extracts of organs removed from animals dying during the acute reaction, and also by inoculation of fluid from the pericardium. The virus has not been recovered from the bile. The urine is infective, but not the fæces.

The virus is relatively resistant. Serum or citrated blood remains virulent for about 28 days in flasks plugged with cotton-wool, and for about 45 days in sealed tubes. The virus may be preserved for a week in equal parts of oxalate carboglycerin solution (potassium oxalate 5 gm., carbolic acid 5 gm., glycerin 1,000 c.cm. and water 1,000 c.cm.). It is not destroyed by heating to 50° C. for 1 hour, but is destroyed when exposed to 60° C. for 5 minutes.

Subcutaneous inoculation of virulent serum or blood produces the disease regularly even in a dose as small as 0·001 c.cm. Positive results are also obtained by intravenous and intraperitoneal inoculation or application of virulent blood to the scarified skin. The administration *per os* of large quantities of blood (50 c.cm.) may produce the disease. Attempts to infect by cohabitation have been negative.

The disease is transmitted by the adults of *Rhipicephalus appendiculatus* which have fed as nymphæ upon reacting sheep.

Animals Susceptible and Animals Resistant to the Infection.

Native-bred sheep are the most susceptible, whereas grade and merino sheep show a greater resistance. Goats can be infected, but they are more resistant than sheep. Cattle are not susceptible, but when inoculated with virulent material their blood may remain virulent during 24 hours. Attempts to infect buffaloes, horses, donkeys, pigs, dogs, rabbits, guinea-pigs, rats and mice have been few in number, and all have yielded negative results.

Immunity.

An animal which has recovered from the natural or experimental disease does not react to a second infection if made within a few months. Acquired immunity may apparently exist for several years in some cases. A recovered animal is not a reservoir for the virus. Attempts to produce an immunity by the use of virus attenuated by heat have not given promising results. Some few experiments have been carried out to produce a hyperimmune serum in sheep, but the resulting serum was found to be hæmolytic. The virus becomes attenuated by passage through goats, and such attenuated virus appears to give some protection to sheep, although the experiments in this direction have been few.

Prophylaxis.

Preventive measures should include the eradication of ticks. *Rhipicephalus appendiculatus* is also the carrier of East Coast fever in cattle, and dipping cattle at 3-days' interval will diminish the number of ticks on an infected pasture.

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CHAPTER XXVII. PLEURO-PNEUMONIA CONTAGIOSA BOVUM.

BY J. WALKER (NAIROBI).

WITH A SECTION ON AGALACTIA BY I. A. GALLOWAY (NATIONAL INSTITUTE FOR MEDICAL RESEARCH, LONDON).

[Bovine Pleuro-Pneumonia; Lungsickness of cattle (British); Lungenseuche der Rinder (German); Peripneumonie contagieuse (French); Polmonera-polmonite essudativa (Italian)].

Bovine pleuro-pneumonia is a contagious disease naturally confined to bovines; it is caused by a filterable virus which produces an exudative fibrinous pneumonia and pleuritis, and specific histological lesions. It is distributed widely throughout the world with the exception of Western Europe, North America and India. Nocard and Roux (1898), with the collaboration of Borrel, Salimbeni and Dujardin-Beaumetz, were the first to demonstrate the causal agent. In 1900 its filterability through Berkefeld and Chamberland F filters, and its cultivation in liquid and solid media were demonstrated by Dujardin-Beaumetz.

The Virus.

Morphology and Staining.

Morphology. Although the morphological appearances described by various workers correspond in some respects, and opinions differ as to the group in which the organism should be classed, it is generally accepted that the causal agent of bovine pleuro-pneumonia presents variations in size and shape giving rise to polymorphism. The rapidity with which the various forms develop in liquid culture, the medium in which it is grown, the age of the culture and the methods employed for examination would appear to explain the dissimilarity.

In view of the morphological appearances as described by Borrel, Dujardin-Beaumetz, Jeantet and Jouan (1910), the scientific name '*Asterococcus mycoides*' was proposed by these investigators, but the choice would appear to be of very doubtful value. They found that the morphology was influenced by the age of the culture and that the various forms succeeded each other more or less rapidly in chronological order; that the spirillum or vibrio form described by Bordet (1910) is only one of the aspects which the organism takes; that the organism is ensheathed within a mucin matrix, and occurs in diplococci, chain and mass formation, in which the individuals are of different size; in the two latter, each unit can divide, originating bifurcations and asteroid forms. Tripolar forms are frequent and, more so, the bipolar or pseudo-vibrio forms. The arrangement of individual organisms in ring formation is frequent, and the ring can lengthen in the form of a filament or chain. In 3 to 4 days' old preparations the pseudo-vibrio form is dominant. There also occur masses of granulations in tetrads and mulberry shape which can bud and give rise to filaments, this stage being the commencement of the asteroid forms. The filamentous forms are constant in 5 to 6 days' old cultures. In very old cultures the more extraordinary forms of involution and coalescence of mucin substance secreted by the older individuals are to be seen. Titze and Seeleman (1921) examined cultures in the dark field and found that the organism presented the following aspect, viz. : numerous roundish refractile bodies of different size showing molecular movement, sometimes two or three lying together as if bound together by an invisible bridge, with the movement in the same direction ; ring forms with clear periphery and dark centre were often visible, while filamentous or branching forms were not seen ; small bodies were also observed in the culture and in sterile Martin's broth and serum, which they conclude are non-specific.

Frosch (1922), working with ultraviolet light, found the organism oval or polygonal in shape, measuring from 0·2 to 0·8μ in diameter, but left its nature undetermined, and in 1923 he described the appearances presented by the organism in a number of different culture media. Orskov (1927) describes the primary form of the virus as a small oval or short rod-shaped element which gives rise to a ramified mycelium. The virus has a tendency to autolyse and form elements which vary in shape and which have lost their power of germination. Smiles (1926) examined 3-day cultures in serum broth by dark ground methods and noted the presence of single spheres or spheres with one attached particle as well as numerous free particles. Elford (1929), using similar methods, reports that in vigorous serum-broth cultures there are two predominant elements: (1) spheres measuring 0·2 to 0·25μ in diameter, and (2) particles exhibiting active movement. By ultrafiltration methods the latter can be separated from the former, but give rise to spheres in culture. The estimated size of these particles is 0·125 to 0·150μ.

Staining. The organism is Gram-negative. In bouillon cultures it stains with aqueous or, preferably, carbolized solution of the aniline dyes. Giemsa stain is preferred by some. Bordet (1910) uses Giemsa stain in the proportion of 5 drops to 2 c.cm. of distilled water, heated on the slide for 1 to 2 minutes. Borrel, Dujardin-Beaumetz, Jeantet and Jouan (1910) found with Giemsa that many of the forms escape detection and are indefinable. They prefer Loeffler's mordant method for studying the morphology, and make smear preparations of the organisms which accumulate at the bottom of the tube on centrifuging a liquid culture.

Cultivation.

Cultivation in vivo.

Nocard and Roux (1898), with the collaboration of Borrel, Salimbeni and Dujardin-Beaumetz, successfully cultivated the organism by inserting in the peritoneal cavity of rabbits collodion sacs containing bouillon inoculated with a few drops of sero-fibrinous exudate collected from the pleural cavity of a naturally infected beast. Although with a high magnification (1,500 to 2,000 diameters), and high illumination a number of small bodies were observed in the contents of the sacs their structure could not be accurately determined, but their specificity was demonstrated by inoculation of susceptible cattle. The use of collodion sacs is not essential, however, and direct cultivation is possible provided a satisfactory broth is employed.

Cultivation in vitro.

Development takes place between 30 and 42° C.; the optimum temperature lies between 36 and 38° C.; below 30° C. no growth takes place.

In liquid media. The medium commonly employed consists of Martin's peptone beef bouillon to which bovine serum brought to pH 7·5 is added. Growth occurs in beef bouillon, or Martin's peptone beef bouillon, containing any one of the following sera: human, horse, swine, sheep, goat, rabbit and fowl or ascitic fluid. Nakamura, Futamura and Watamiki (1926) prepared broth from beef, pork, horse and rabbit flesh, but cultural experiments failed to reveal any marked superiority of these. The addition of 0·5 per cent. glucose increases growth.

The addition of serum to the medium is essential. With less than 5 per cent., growth is usually scanty. The writer adds 10 per cent. of fresh bovine-serum, or horse-serum, previously sterilized by filtration through a Berkefeld filter. Titze, Giese and Wedemann (1923) found that in a liquid medium containing 7 to 10 per cent. horse-serum, the organism develops quicker and better than in a medium containing bovine-, sheep- or goat-serum. They conclude that in a Martin's peptone bouillon serum with an acid reaction, growth scarcely occurs, in a neutral Martin's peptone bouillon serum only a scant growth, while in distinctly alkaline medium the growth is better than in a weak alkaline medium.

In cultures *Asterococcus mycoides* develops best under aerobic conditions, but the writer finds that a free access of air is not indispensable, that no appreciable influence on growth occurs when bottles containing Martin's peptone beef bouillon bovine-serum are corked and sealed with paraffin wax immediately after inoculation of the medium with pure culture and then incubated.

Character of the culture in Martin's peptone beef bouillon (pH 7·5) to which 10 per cent. bovine- or horse-serum is added. Flasks of the medium, proved for sterility, are inoculated with a drop or two of pure culture and incubated at 37° C.; any which show a growth within 36 hours are discarded. After 2 or 3 days' incubation at 37° C., a slight opalescence appears in the inoculated culture medium; during the course of the next day or two the opacity becomes more marked and on shaking the tube of culture a characteristic phenomenon is visible, namely, the opalescent fluid takes the form of silk-like moving waves.

Walker (1921[1]) prepares pure culture vaccine in bulk by the above-described method.

Cultivation on solid medium. The medium commonly employed consists of Martin's peptone beef bouillon serum-agar, or beef bouillon serum-agar. The medium is first added to the tubes, and the tubes are then laid in a horizontal position until the contents solidify. The water of condensation is removed with a pipette. The serum is then added to the surface of the slope, and the tubes incubated for two days in such a position that the serum spreads over the surface and dries. Other workers prefer to incorporate the serum with the agar before solidification.

After 4 to 5 days' incubation at 37° C., small single colonies visible with the hand-lens are to be seen. With a low-power lens, fine transparent separate colonies which individually have been described as resembling a drop of dew appear on the surface of the slope. They measure approximately 2/10th mm. in diameter, and have a granular and brownish appearance and a dark central portion. The growth extends outwards from the periphery of the colonies, which reach a diameter of 1 mm. The colonies have an *umbilicate* appearance, the central portions growing down into the substance of the medium. With the naked eye, the colonies appear whitish. Difficulty is experienced in removing them from the medium owing to their being held together by a viscid material, and to growth extending into the medium.

Biochemical Reactions.

Action on carbohydrates. Glucose, maltose, dextrin and lævulose are fermented, but not lactose, saccharose, mannitol, dulcitol, arabinose, raffinose, rhamnose, salicin, xylose, adonitol, galactose, inulin, mannose or sorbitol. Gas is never produced.

Indole formation. Indole is not produced in cultures.

Biology.

Vitality, virulence, and resistance. On referring to the literature it is observed that usually no distinction is clearly made between the vitality and virulence of the virus.

Asterococcus mycoides does not retain its vitality for more than a few weeks in liquid culture in tubes plugged with cotton-wool and kept at room temperature excluded from strong light. In liquid media containing lævulose or maltose, and particularly in glucose, more marked acidity occurs, and the cultures become inactive sooner than in liquid media not containing sugar. Dujardin-Beaumetz (1913) records that it is difficult to obtain a growth from a culture in liquid medium after 4 weeks, and when the medium contains glucose, after 3 weeks. Titze, Giese and Wedemann (1923) found that a 27-day old culture in Martin's peptone bouillon of pH 5·8 containing grape-sugar produced a characteristic growth, whereas a 41-day old culture of pH 5·8 had lost its virulence. Dahmen (1922) found that cultures on solid medium were viable up to 2 months.

Titze, Giese and Wedemann (1923) obtained a growth from cultures in Martin's peptone beef bouillon containing bovine-serum, kept at room temperature in the dark in tubes plugged with cotton-wool, for 59 days, but not in cultures kept for 80 days; in tubes plugged with cotton-wool sealed with paraffin wax, and in tubes hermetically sealed in the flame, cultures were still active at 6 weeks. A 62-day old culture, in Martin's peptone bouillon serum on which liquid paraffin was placed on the surface of the culture, produced a characteristic growth in 48 hours.

Nakamura, Futamura and Watamiki (1926) record that cultures kept at 37 to 38° C. had lost their virulence in from 1 to 2 months; at 5 to 32° C. the virulence was maintained for 2 to 4 months, and at 3 to 15° C. for a little longer; variations occurred in different cultures and it was necessary to transplant cultures once a month. They found that pleural exudate kept in the ice-chest at 3 to 15° C. retained its virulence for 6 months. The writer found subcultures in Martin's peptone beef bouillon bovine-serum which had been incubated for 9 days and then stored for 21 days at room temperature in the dark still virulent.

The determination of the period during which a culture retains its virulence is of practical importance when pure cultures are used for immunization purposes.

Lymph virus. Laquerrière (1890) found that the virulence was maintained for, at least, a year in hepatized lung kept in a frozen condition. Walker (1921^2) found lymph virus to which glycerin had been added in the proportion of 1 of glycerin to 4 of virus was effective up to, at least, 21 days when stored in the dark at ordinary room temperature.

Titze, Giese and Wedemann (1923) recovered the virus by filtration from material which was in a putrid condition for 9 days.

Attenuation. Nocard and Leclainche (1903) state that the serous exudate when collected pure and sealed in glass tubes conserves its virulence for about 1 month, and the virulence then decreases; in contact with the air and light the virus was already very attenuated after 20 to 25 days.

Walker (1921^2) found that the original culture and the first and second generations of subculture are as virulent as the virus from which they originated; at about the twenty-fifth generation of subculture the virus is attenuated and produces less marked reactions; from about the twenty-fifth to the forty-second generation of subcultures, the virulence remains constant. The virus, though retaining its vitality, eventually becomes so attenuated in virulence that it produces few, if any, reactions, and it is necessary to discontinue the use of the too attenuated pure culture vaccine and substitute a more virulent subculture of another strain, or increase the virulence of the same strain by passage through susceptible cattle before it becomes too attenuated.

Desiccation. Titze, Giese and Wedemann (1923) soaked silk threads and glass beads, respectively, in a 48-hours' old culture and dried them for 60 hours over calcium chloride at room temperature in the dark;

the former produced a visible growth in 48 hours, the latter in 96 hours, in Martin's peptone beef bouillon serum. After being kept in a dried state in sealed tubes for 5 months the virus was found still active in the silk threads, but only on some of the glass beads. Pieces of cambric cloth soaked in culture and then dried at 37° C. for 12 hours, as well as pieces dried at 37° C. for 15 days, produced a growth in Martin's bouillon serum, whilst after 36 days the virus was inactive. In a bouillon culture dried in vacuum, over calcium chloride, the virus was still active on the hundred and fifth day.

Influence of dry heat. The organism produces no spores, it is little resistant to heat either in tissue or in culture. The cultures are readily destroyed in less than an hour by heating at a temperature of 58° C.

Infection.

Natural Infection in Cattle.

The history of the spread of bovine pleuro-pneumonia supports the generally accepted view that it is contracted by immediate contact with infected animals; the virus is present, at least in some cases, in the bronchial secretions and nasal discharge, and in the moist exhaled air. The virus of bovine pleuro-pneumonia in all probability gains entrance with the inhaled air. Transmission by an intermediate host has not been established. Outbreaks can usually be traced to the introduction into the herd of an infected animal which presented no clinical symptoms.

Walley (1913) found that the virus exists in the encapsulated cavities for 15 months. Minette (1913) traced outbreaks in herds to cattle introduced therein 2 and 3 years previously. Walker (1923) found the virus active, in some instances, 12 months after the animals were isolated as infected.

Observations show that a variable number of susceptible cattle exposed in immediate contact with naturally infected beasts escape infection, but the cause of these variations has not, so far, been explained. Nocard and Leclainche (1903) suggested that they might be due to differences in the activity of the virus and to the resistance of the animal. Walker (1922) found that approximately 58 per cent. of non-inoculated susceptible cattle exposed to natural infection in a highly infected herd, for periods varying from approximately 3 to 7 months, did not contract infection. Further research is considered necessary hereon.

Experimental Infection in Cattle.

Infection by the respiratory tract. Although infection by inhalation is supported to some extent by the ingenious experiments of Nocard and Roux (1901) and Chauveau (1903), it has not been possible to produce artificially, by inhalation or injection of virulent material, the disease as it occurs under natural conditions.

Subcutaneous inoculation. In about 6 to 14 days after subcutaneous inoculation behind the shoulder of a virulent culture or lymph virus, there occurs in a varying percentage of susceptible cattle a rise of temperature and an œdematous swelling, which, in some cases, extends considerably, resulting in the death of the animal in 10 to 15 days. In other cases the swelling remains localized and gradually disappears. In sucking-calves subcutaneous inoculation often produces specific lesions in the joints and tendons, viz. synovitis and tendovaginitis.

Infection by the alimentary canal. Nocard and Roux (1903) showed that ingestion of large quantities of serous exudate gave negative results provided the virus did not enter a defect in the tissues. At one time in South Africa drenching with lung exudate was employed for immunizing purposes, but it was discontinued owing to the accidents which followed.

Intravenous inoculation. Nocard and Roux (1903) inoculated virus into the ear vein and immediately afterwards removed a portion of the ear around the site of inoculation, so as to exclude the virus developing in the subcutaneous tissue. No reaction occurred and no immunity.

Inoculation into the peritoneal cavity produces a marked reaction and a sero-fibrinous exudate usually ending fatally.

Intracerebral inoculation of culture, after an incubation period of 6 to 14 days, produces apathy and drowsiness interrupted by symptoms of cerebral irritation. Animals refuse food and there is a rapid loss of condition ; death occurs in a variable number of days.

Inoculation into the udder produces a marked mammitis ; within a few days the milk becomes yellowish, caseous and purulent. The œdema extends to the abdomen and may produce death ; the pus-like milk contains the virus even after two months and the virus is said to be increased in virulence.

Intrapleural inoculation is followed by an intense inflammation ; the lung itself may show hepatization ; the peritoneum may become involved by way of the lymph vessels.

Experimental Inoculation in Species naturally Refractory.

Dujardin-Beaumetz (1906) conceived the possibility of experimentally infecting sheep and goats with a virus of bovine origin cultivated in Martin's peptone sheep bouillon containing sheep-serum, and succeeded in obtaining in sheep and goats by inoculation of from 50 to 100 c.cm. of the culture a local and temperature reaction like that which occurs in bovines inoculated with lymph virus, or cultures of virus of bovine origin in Martin's peptone beef bouillon ox-serum, but with this difference, that there was no incubation period in the sheep and goats. He found that cultures in Martin's peptone bouillon sheep-serum possessed a high pathogenicity for the bovine, but virus collected from a bovine reacting to inoculation of a culture in bouillon sheep-serum, when cultivated in bouillon with ox-serum, is non-pathogenic for the sheep and goat. It thus sufficed to substitute the serum of one species for another to overcome the resistance of the naturally refractory species. This investigator also found that the culture in media containing serum of other naturally refractory animals, e.g. the horse, produced a reaction in naturally refractory species, viz.: sheep and goats, but not in highly susceptible species (bovine), and he suggested for the preventive inoculation of cattle the utilization of a vaccine prepared from cultures of virus of bovine origin in Martin's peptone beef bouillon containing horse-serum, with a view to conferring immunity without the risk of losses from inoculation.

Beller and Tahssin-Bey (1926) thought it desirable to test the possibility of the transmission of bovine pleuro-pneumonia to small ruminants, because of the possibility of some parallel existing between the spread of pleuro-pneumonia and rinderpest. Both sheep and goats were found capable of infection and they concluded that, in controlling the disease, the sheep and goat must be kept in mind as possible carriers of the virus.

Reindeer. According to Dschunkowsky (1901) the reindeer can be experimentally infected by subcutaneous inoculation of bovine virus.

Other animals. Willems (1852) found that rabbits, dogs, fowls, sheep, goats, and swine, do not react to inoculation of bovine lymph virus.

Ono (1925) recorded that intra-ocular inoculation of rabbits produces an iritis while intratesticular inoculation produces a swelling lasting for 2 to 3 weeks; there was no suppuration. Serum of the inoculated rabbits gave a precipitin reaction with serum from infected cattle. Walker (1921^2) found that subcutaneous and intraperitoneal inoculation of virus to small laboratory animals, mice, guinea-pigs and rabbits, and the subcutaneous inoculation of the sheep, the horse, the camel and swine, as well as the intrajugular inoculation of sheep, produced no reaction.

Pathogenesis.

Different workers have investigated the pathogenesis of the processes which occur consequent on the entry of the virus into the lungs with the inspired air. The conclusions arrived at as regards the primary seat of infection in the lungs, the spread of the virus therein and the first tissue changes differ considerably, and those interested are referred to communications by Sussdorf (1879), Pourcelot (1881), Woodhead (1888), MacFadyean (1892), Csokor (1898), Meyer (1909), Ziegler (1921) and Seifried (1926). It would appear to be agreed that the virus on entrance to the lung multiplies in the lymph-vessels of the interlobular connective tissue. The inflammatory process spreads to the neighbouring lobules and to the alveoli. The lymph spaces of the blood-vessels of the alveoli finally become involved, leading to vascular thrombosis with consequent production of a form of ischæmic lung necrosis and sequestrum formation.

Arloing (1913) found a sequestrum contained within a connective tissue capsule as early as the fiftieth day, while Walker (1924) observed sequestration and encapsulation taking place in from 15 to 22 days. The sequestrum, after becoming encapsuled, undergoes mummification and remains unaltered for a long time, during which the virus remains active; abscess formation and liquefaction may also occur.

The virus does not propagate in the blood-stream, but may be transported thereby to different parts of the body, viz.: in calves to the joints and tendons; metastasis of the virus from the seat of inoculation may occur to other parts of the body, e.g. the sites of surgical operations.

Naked-eye features. It is usual to find only one lung affected. In acute cases the hepatized portion, which is as a rule conspicuous on account of its roundness and firm consistence, may be localized in a portion of a lobe or invade almost the whole of a lobe.

The pleura is sometimes covered with a fibrinous membrane and there may be no exudate present (pleuritis sicca). In other instances deposits of 1 to 2 cms. thick are present on the surface, and the pleural cavity contains a clear yellowish or reddish-yellow liquid, varying in quantity from 1 to 2 litres up to 20 litres. In older cases the pleura is thickened and the lung firmly adherent to the chest wall.

Symptoms.

Incubation period. This is very variable, but symptoms may appear under natural conditions in from 3 to 6 weeks with a minimum of 10 days after contact.

It is possible to distinguish, according to the rapidity of evolution, three forms, viz.: an acute, a peracute and a subacute.

Acute form. In the early stages there are no significant symptoms, but within the next few days symptoms become marked. The animal is disinclined to move, the skin is dry, hair erect, respirations accelerated, the temperature becomes elevated and may reach as high as 104° F. or over, and milk secretion diminishes. The local symptoms now manifest themselves; there is a short, dry, painful cough which is provoked by percussion of the chest wall. The symptoms now become intensified. The animal is disinclined to move and stands with the head extended and elbows turned outwards; temperature remains elevated; there is a partial anorexia; during inspiration the nostrils are widely opened, the stronger extension of the thorax is followed by a double contraction of the abdominal muscles, the cough becomes more frequent, moist and painful, expiration is accompanied by a groan. Percussion of the chest wall indicates usually in the lower parts of the affected lung, behind the shoulder and up to a certain height, a partly or entirely dull sound, limited above by a horizontal line (pleural exudate) or a zone of dullness and hepatization. Auscultation at first shows weakened vesicular breathing and some crepitation in the anterior and lower parts; later, in the region of the partially or entirely dull area no vesicular or bronchial breathing may be heard, but this is usually perceptible around the borders of the affected area.

In the later stages the animal is exhausted, and muscular tremors occur. There is usually a muco-purulent discharge from the nostrils, and the respirations become discordant. An œdematous infiltration of the dependent parts of the chest and abdominal wall is often present.

In this form the evolution is complete in from 2 to 3 weeks. In a percentage of cases the disease comes to a standstill, and the symptoms gradually disappear; though the cough persists and becomes stronger, respiratory difficulties subside. The appetite, rumination and milk secretion return to the normal, the animal gains condition, and after the lapse of a varying period of time appears to have recovered, but it remains a source of infection for a varying period.

In animals kept housed and well fed, the percentage of recoveries is greater than in cattle exposed under adverse field conditions.

Peracute form. Exceptionally the disease may have an acute onset with severe symptoms, the lesions develop with a sharp rise of temperature. The symptoms differ according as the pleural or pulmonary lesions predominate. When the pleurisy is intense, percussion and auscultation indicate the presence of an abundant exudate; when the pulmonary lesions prevail, the cough occurs from the commencement. Termination by asphyxia is the rule; death occurs in from 2 to 8 days.

Subacute form. Sometimes the lesions remain localized in a small part of the lung; in such cases the only symptoms may be a rare cough, and percussion and auscultation do not furnish any precise indication. Sometimes new foci of infection occur in the lung and the animal shows acute symptoms, but usually the lesions disappear and the disease escapes detection.

Chronic form. A chronic type is also described in which the only symptom manifested is cough on disturbance after resting.

Diagnosis.

In the Living Animal.

Diagnosis, particularly of the first case or two of an outbreak, cannot be assured by clinical examination; if the symptoms are suspicious, the history of the movements of the cattle belonging to the herd and the possibility of an infected beast having been introduced should be investigated; serological methods of diagnosis could be employed if facilities exist for such, but slaughter of a suspect beast for post-mortem examination and collection of material for pathological-anatomical and histological examination and cultural tests is desirable.

Pathological-anatomical and Histological Diagnosis.

The microscopic lesions, viz.: exudative pneumonia and pleuritis and sequestrum formation, are as a rule characteristic, yet they may sometimes be confused with other pneumonias, e.g. the pectoral form of hæmorrhagic septicæmia of bovines; on the other hand, specific histo-pathological changes are usually demonstrable.

Meyer (1909) found that the thick rings of leucocytes with deposits of fibrin which surround the blood-vessels of the interstitia in circular symmetrical arrangement can be used with certainty for diagnosis. Ziegler (1921) concludes that necrosis and the perivascular and marginal organization processes in the interstitia and the parabronchitic changes are specific.

Cultural tests. The pulmonary or pleural exudate is diluted in Martin's peptone beef bouillon medium in the proportion of 2 of exudate to 98 of the medium, and 10 per cent. of filtered bovine serum added. The tubes are incubated at 38° C.; if the fluid becomes opalescent in 3 to 4 days and microscopically there is no distinguishable bacterial infection, the diagnosis may be considered as established.

Biological tests. The impossibility of detecting by clinical means animals with latent lesions, the so-called ' Lungers ', and the role which they play in the maintenance and spread of infection, prompted workers to determine the value of certain biological tests for diagnostic purposes, viz.: agglutination, complement fixation, conglutination reaction and precipitation methods. In addition to the above the lipoid fixation (Meinicke Bley) and the flocculation reaction of Sachs and Georgi have been investigated. None of the methods are infallible, but the percentage of errors which occur with one may possibly be reduced by the simultaneous application of another. Their practical value is reduced owing to the necessity of repeating the tests at intervals so as to exclude the risk of missing animals in the incubation stage of the disease and others which may have contracted the disease after the date of the original test. Should the test fail to reveal all infected animals and restrictions of movements of the herd be removed, serious consequences are likely to occur, particularly in the case of trade and transport cattle, which may originate widespread foci of infection.

Agglutination. Although agglutinins have been demonstrated in the serum of some naturally infected or hypervaccinated cattle, and the agglutination method is of some assistance, yet the percentage of errors is too high for it to be used alone.

Complement-fixation method. This is of some practical value. Titze and Giese (1919) and Titze, Giese and Wedemann (1923) recorded satisfactory results, but found that hæmolysis subsequently occurred in the tubes which showed a fixation, thus necessitating the reading of the tests within a time limit. This difficulty was, however, overcome by using culture antigen. Heslop (1921, 1922) concluded that the method is not sufficiently accurate to warrant its general use, the main difficulty being to prevent errors owing to the hæmolysis which occurs consequent on the presence of conglutinin and some excess of complement. Dahmen (1922, 1923) recorded observations on the value of various antigens; Nakamura, Futamura and Watamiki (1926) found with a suitable antigen the complement-fixation method reliable. Walker (1923) obtained practical results and found that the hæmolysis is eliminated by the use of the conglutination method.

Precipitation method and its modification. Precipitin and precipitinogen have been demonstrated in the serum of infected cattle, and the latter in extracts of hepatized lung. The percentage of errors limits the practical value of the methods.

Lipoid fixation (Meinicke Bley) and Sachs and Georgi flocculation reactions. Titze, Giese and Wedemann (1923) obtained unsatisfactory results and were unable to express an opinion on the practical worth of these methods.

Allergic reaction. Siedamgrotzky and Noack (1892), Walter (1892) and Beitzen (1919) carried out diagnostic inoculation with sterilized pulmonary exudate, and Titze, Giese and Wedemann (1923) with concentrated sterilized culture. They obtained temperature reactions in a percentage of infected as well as non-infected cattle. The test is not considered of sufficient practical value to introduce into practice. Since results depend on a temperature reaction, it would be of less value in tropical countries owing to the variations in temperature which occur in healthy non-housed cattle. The last-named workers found the intrapalpebral and ophthalmic infection with concentrated pure culture of no assistance.

Control Measures.

These comprise preventive inoculation, slaughter of affected animals, quarantine and in addition serum-diagnosis tests if facilities for such exist. Preventive inoculation has been made much use of since Willems (1852) demonstrated that inoculation with pulmonary or pleural sero-fibrinous exudate (lymph virus) confers a resistance against natural infection and re-inoculation. The method, however, is probably of much older date. The Arabs and Moors of North Africa have practised from time immemorial the rubbing of pleural lymph into the scarified skin of the forehead. Pure culture vaccine is now generally substituted for lymph virus where facilities exist for its preparation. Walker (1921) issues an attenuated pure culture vaccine.

In some countries, Great Britain, United States of America and in various European countries, preventive inoculation, slaughter of affected animals and quarantine measures were found insufficient, and extreme measures, e.g. the slaughtering of infected animals and contacts were found necessary to control and eradicate the disease. South Africa was officially declared free of bovine pleuro-pneumonia in 1914. The measures adopted there consisted of preventive inoculation, slaughter of those visibly affected and quarantine of the herd for three months. In the writer's experience a large number of infected cattle recover and are no longer a source of infection, and this fact would appear to explain, at least partially, the successful eradication of the disease in South Africa. In countries in which the disease is widespread, generalized periodical preventive inoculation should be of assistance in reducing its incidence when slaughter of the visibly affected cannot be considered. In the case of isolated outbreaks slaughter of infected animals and contacts is usually desirable.

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Agalactia.

BY I. A. GALLOWAY.

Synonyms: Agalaxie contagieuse, Mal dell' asciutto, Agalactie infektiöse.

Description.

Agalactia is an infectious and contagious disease of sheep and goats characterized by inflammatory lesions in the mammary gland, the eye and the articulations. In animals infected when lactating, a serous, dirty yellow fluid, with small clots, is secreted in place of milk. The disease is caused by a small pleomorphic micro-organism, resembling that of bovine pleuro-pneumonia, which is capable of passing through a Berkefeld, Silberschmidt or Chamberland L I (bis) filter.

Epizootiology.

Metaxà (1816) first described the disease under the name of 'Stornarella' as occurring in Italy. Since then, numerous strictly localized epizootics have been reported, e.g. in Switzerland, the Tyrol, North and Central Italy and various departments of France. Sergent and Roig (1917) described a small epizootic which occurred in Algeria in 1908, and which was believed to have originated from the importation of infected goats. Later, Bridré and Donatien (1925) found that sheep bought for the Pasteur Institute there in 1923 were infected. Thousands of sheep and goats may be affected in such epizootics. In 1896 over 43,000 were reported to be suffering from agalactia in the Province of Aquila alone. Carré (1912[2]) described an epizootic in the region of Lure in which acute pyæmia ('Mal de Lure ') was a leading feature.

The mortality averages about 15 per cent. Epizootics are commonest in the spring, may also occur in the summer, but disappear in the winter.

Ætiology.

The disease is contagious, and Carré (1912[1]) believes ingestion to be the most usual method of natural infection.

In the earlier work various investigators isolated micro-organisms from the lesions, including streptococci, staphylococci and bacilli, but with none of these could agalactia be produced on inoculation, although many of the secondary lesions may be due to such organisms. Celli and de Blasi (1896) showed that the disease may be reproduced by inoculation of the altered milk, subcutaneously. In 1906 they found that such fluid retained its infectivity after filtration through a Berkefeld or a Silberschmidt candle. Carré (1912[1]) confirmed their findings. It is now known that susceptible animals may be infected by inoculation subcutaneously, intraocularly, into the joint, intraperitoneally, intramuscularly or intravenously, of the altered milk, the arthritic fluid or scrapings from the cornea. Feeding with infective material, direct infection into the milk ducts, or simple contamination of the teats of lactating animals also produces the disease. After introduction of virulent material into the mammary gland, the milk becomes altered on about the third or fourth day. The infection may remain localized, or 12 to 13 days later may be followed by generalized lesions in the eye or articulations. It is easiest to demonstrate the virulence of suspected fluids by inoculation into lactating animals, and especially goats.

Although various investigators believe the infective power of the secretions of infected animals to be at a maximum, or only present, during the early stages of infection, Carré (1912[1], 1921) showed that the fluid expressed from the mammary gland may still be infective 6 months, and even 8 months, after the animals first show symptoms of the disease. He also showed that the tears may be infective, although there is no open lesion of the eye. Marra and Cocciante (1912) found the blood to be virulent at the time of the temperature reaction. Carré (1912[1]) showed that about 500 to 800 c.cm. of virulent material could be obtained by the inoculation of virus into the pleural cavity with gluten casein. Celli and de Blasi (1906) stated that virulent material kept at 14° C. in the dark was inactive after 2 months.

Bridré and Donatien (1923) recovered from the fluid of an inflamed articulation of an infected sheep a very small pleomorphic micro-organism, staining by Giemsa's stain and resembling that of bovine pleuro-pneumonia. This micro-organism can be recovered in the purest state from arthritic fluid or from lymph glands draining infected organs. If necessary such fluid can be separated from secondary invaders by filtration. The micro-organism will grow in Martin's bouillon with the addition of serum in the proportion of 1/5 to 1/10. The serum of sheep, goats, horses, oxen, asses, rabbits and man can all be used. It will also grow in serum with peptonized water in the proportion of 1 to 2. The addition of 1 per cent. solution of certain sugars such as lactose and mannitol seems to favour growth. In a medium consisting of Martin's bouillon and the serum of an hyper-immunized animal it grows well, but such cultures are no longer infective nor do they immunize. With fresh material there is no apparent growth for 3 to 4 days, but after passage growth is visible after 24 hours. The optimum temperature is 37° C., but growth takes place at any temperature between 24 and 41° C. In culture, all the forms which have been described for the organism of bovine pleuro-pneumonia may be found (see Bordet, 1910; Borrel, Dujardin-Beaumetz, Jeantet and Jouan, 1910). The virulence of cultures is as great as that of pathological products. Under aerobic conditions at 37° C., cultures are avirulent at the end of a month, but under anaerobic conditions they are still virulent even after 22 months. At lower temperatures, cultures die out more rapidly. At 0° C. cultures are dead after 3 months. In the ice-chest at a temperature varying between —5 and —12° C. cultures were found to be still viable after 4 months, but not after 5 months.

The micro-organism will grow in goat's or cow's milk, and such growth produces no change in the medium. On serum-agar, colonies are obtained similar to those which have been described for the micro-organism of pleuro-pneumonia. The micro-organism is killed by heating to 50° C. for half an hour, and to 53° C. for 10 minutes; 1 in 10,000 copper sulphate prevents growth, while greater dilutions have no inhibitory action. Carbolic acid in a dilution of 1 in 1,000 also kills the organism. The subcutaneous inoculation to 0·5 to 1 c.cm. of a broth culture produces a local reaction in 4 to 7 days, and generalized lesions in 1 to 4 weeks. Intravenous inoculation of cultures gives a severe reaction which is invariably followed by death. The inoculation of the micro-organism into the articulations produces an arthritis. Infection can also be produced by scarification of the skin.

Animals Susceptible and Animals Resistant to Infection.

Goats and sheep are susceptible, the former more so than the latter. Lactating animals are the most susceptible, but lambs, males, and non-lactating animals may also be affected. Young animals are less susceptible than adults. The disease can pass from the mother to the fœtus. Under natural conditions and experimentally, only a certain percentage of the animals contract the disease. Some acquire an immunity by a mild attack, while others are not affected, but are still susceptible. Attempts to infect cattle have been made by Carré (1921) and Bridré and Donatien (1925) with doubtful results. Experiments on small laboratory animals have been few in number. Celli and de Blasi (1906) stated that a parenchymatous keratitis was produced in the rabbit by inoculation of a filtrate into the anterior chamber. Sergent and Roig (1917) could not infect the rat or the guinea-pig by inoculation of virulent material intraperitoneally.

Immunity and Chemotherapy.

Animals which have recovered from a spontaneous attack have a solid immunity. Bridré and Donatien (1925) showed that such recovered animals would resist an intra-articular inoculation of 0·2 c.cm. of a serum broth culture without showing any symptoms. The young of hyperimmunized animals are also immune.

Carré (1912[1]) hyperimmunized animals recovered from the spontaneous disease by repeated subcutaneous or intravenous inoculation of virus in the form of pleural exudates. He found that the sera from these animals protected ewes against a strong test dose of virus administered intramuscularly or by the mouth. A mixture of hyperimmune serum and virus would also protect ewes against a subsequent test dose administered by the subcutaneous or intramuscular route. Later (1921), he showed, however, that the immunity induced by sero-vaccination, even when reinforced by a dose of living virus, would not protect against direct infection of the mammary gland. As a result of this observation he suggested local vaccination of that organ, but this does not appear to have been attempted. Bridré and Donatien (1925), using cultures, also prepared hyperimmune sera from sheep, goats and horses. Such sera protected lactating goats against a subcutaneous, but not an intra-articular inoculation of a test dose of culture. They were also persuaded from certain experiments that hyperimmune serum would localize and limit generalization in infected animals. The experiments of Carré (1921), who used a ‘ sensitized ’ vaccine, and Bridré and Donatien (1925), who used sub-infective doses of non-attenuated cultures and cultures attenuated by various means, show that the production of an active immunity is extremely difficult, especially in lactating animals. It appears to be easier to immunize the sheep than the goat. In none of their experiments did they succeed in actively immunizing lactating goats.

Bridré, Donatien and Hilbert (1928), in a small number of experiments, obtained good results with stovarsol. Repeated maximum doses of 0·03 gm. per kgm. of this drug appeared to have a preventive and curative action.

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CHAPTER XXVIII. BORNA DISEASE.

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Synonyms. Enzootic encephalomyelitis, Meningo-encephalomyelitis, Névraxite enzootique, Mal d'Azeau (after the region where a severe epizootic occurred in Belgium in 1909); Bornasche Krankheit, Genickstarre, Gehirnrückenmarksentzündung, Gehirnrückenmarkseuche, Nervenfieber, Encephalitis Lymphocytaria.

Description of the Disease.

The name 'Borna disease', by which the disease is more commonly known, originated from the locality in Saxony where a severe enzootic occurred in the years 1894 to 1896. It is infectious, and the characteristic symptoms are due to microscopic lesions in the central and peripheral nervous system, which are produced by an ultramicroscopic and filterable virus.

Clinical symptoms. The period of incubation in the spontaneously contracted disease is difficult to estimate, and is, therefore, not definitely known. The onset may be sudden, but fatigue, gastro-intestinal disturbances, and symptoms of infection of the upper respiratory passages may precede the onset of characteristic nervous symptoms. The first symptom which usually draws attention to the infected animal is lassitude. A period of excitability follows, which may last with intermittence till the end of the disease. Tonic contractions of diverse groups of muscles occur, and there is difficulty in mastication and deglutition. During the stage of excitability, external stimuli produce exacerbation of the spasms in certain groups of muscles, champing of the jaws is a common symptom, and saliva flows from the commissures of the mouth. Soon paralysis or paresis sets in, affecting the hindquarters, the muscles of the tail, muscles of mastication, muscles of the tongue and of the back to a varying degree. The temperature is variable. According to Hutyra and Marek (1922), it often rises at the outset only, and is normal throughout the course of the disease. Although high temperatures have been recorded, according to these authors persistent fever indicates secondary complications. In some cases there is a moderate leucocytosis. Occasionally the cerebrospinal fluid shows a slight lymphocytosis. The duration of the disease varies from a few days to six weeks.

Epizootiology.

Borna disease has been known for more than a century. Probably Wörz was the first to describe the disease in 1813 in Wurtemberg; later, cases of encephalomyelitis have been reported in many parts of the world (see Nicolau and Galloway, 1928[1]). The epizootics have been of varying severity. The mortality is never below 75 to 80 per cent. Epizootics generally begin in the month of May and decline later, the disease becoming sporadic during the winter months. In certain districts the disease is especially prevalent, occurring every year, or at longer intervals.

Relation to Enzootic Encephalomyelitis in Sheep and Cattle.

In cattle and sheep, outbreaks of disease characterized by symptoms of encephalomyelitis were described towards the end of last century, and in some instances the co-existence of such cases with Borna disease was recorded. Recently, comparative studies have been made of equine, bovine and ovine encephalomyelitis (vide immunity) which strongly suggest that varieties of the same virus are the cause of these diseases.

Ætiology.

The disease was attributed by the earlier investigators to the pathogenic action of various cocci, but the recent work has shown that these must now be considered as secondary invaders.

The work of Zwick (1925), Zwick and Seifried (1924, 1925), and Zwick, Seifried and Witte (1926), Beck and Frohböse (1926), Miessner (1926), and Ernst and Hahn (1926, 1927) in Germany, has furnished evidence that enzootic encephalomyelitis is produced by an ultramicroscopic organism. This evidence may be summarized as follows : A virus has been recovered from horses, sheep and cattle ill from spontaneously contracted enzootic encephalomyelitis and passaged through rabbits by intracerebral inoculations. The virus, originating from horses, passaged through rabbits, can be transmitted back to the horse. The virus taken directly from the horse, or subsequently passaged through rabbits, is pathogenic for lambs. Attempts to transmit the disease from sheep to horses have so far been unsuccessful. It is probable that Moussu and Marchand (1924), and Moussu (1926), were working with an infective agent which differed from those isolated by workers in Germany.

Animals Susceptible.

Rabbits, guinea-pigs, rats, mice, fowls and the monkey (*Macacus rhesus*) are all susceptible. In the rabbit the period of incubation varies from 15 to 50 days, after inoculation by the intracerebral route ; a recurrent form of the disease has also been recorded. Generally, rabbits weighing less than 1,500 gm. are more susceptible than older rabbits, but none has ever been found to be naturally immune.

Guinea-pigs are susceptible to infection by the intracerebral route. Death follows in from 3 weeks to 13 months, and there is great variation in individual susceptibility. It has not been found possible to infect guinea-pigs by intradermal inoculation of an emulsion of virulent brain into the metatarsal pad (Nicolau and Galloway, 1928[1]). The virus passaged through guinea-pigs still preserves its pathogenicity for the rabbit.

Zwick, Seifried and Witte (1926), succeeded in infecting rats by intracerebral inoculation. Nicolau and Galloway (1927 and 1928[1]) are of the opinion that young rats are less susceptible than old ones, and that virus passaged through rats when inoculated intracerebrally into rabbits produces the disease after a shorter incubation period than when the virus is passaged in series through rabbits. In rats death may follow intracerebral inoculation in from 20 to 82 days or longer. They also succeeded in infecting mice by the intracerebral route. While mice are apparently less susceptible to infection than rats, age, as in rats, appears to have an important influence. Mice of more than 20 gm. weight generally contract the disease and die, while smaller mice survive without showing symptoms.

Zwick, Seifried and Witte (1926) found the fowl to be susceptible to intracerebral inoculation. Passage from fowl to rabbit gave a positive result.

Nicolau and Galloway (1927, 1928[1], and unpublished observations) have succeeded in infecting eight consecutive monkeys (*Macacus rhesus*) by the intracerebral route. The period of incubation has averaged 55 days, and the time between infection and death has averaged 89 days. The virus after passage through the monkey does not lose its virulence for the rabbit or guinea-pig. Later Zwick, Seifried and Witte (1927) succeeded in infecting one monkey (*Macacus rhesus*) out of four. They did not record transmission of the virus from monkey to monkey.

The dog, pigeon (Zwick, Seifried and Witte, 1926), cat and ferret (Nicolau and Galloway, 1927, 1928[1], 1930) appear to be resistant to infection.

Modes of Infection.

Intracerebral inoculation produces the disease in a constant manner, followed by death in all susceptible animals. It is also possible to infect animals by introduction of virus intrathecally or into a peripheral nerve such as the sciatic. When virus is inoculated into the anterior chamber of the eye, the typical syndrome and death follows in about 75 per cent. of cases. Scarification of the cornea of rabbits with a virulent emulsion produces no visible keratitis, but on occasion the virus may ultimately reach the brain and lead to infection and death, or if the amount of virus which travels to the central nervous system is small, immunity may follow. Application of virus to the depilated, shaved and scarified skin of rabbits does not usually lead to infection unless the animals so treated have been previously ' prepared ' by inoculation of sterile saline or broth into the brain. Infection of the rabbit may also follow the inoculation of a virulent emulsion into the testicle. A single subcutaneous or intravenous inoculation with virulent material does not produce a fatal encephalomyelitis in the rabbit, but repeated inoculations may do so. As a rule, however, such repeated inoculations lead to immunity. In the same way the intraperitoneal inoculation of virus has failed to lead to successful infection in the rabbit, while the introduction of a large amount of virus by this route may produce a refractory state in the animal. Rabbits can be infected by the inoculation of virulent emulsions into the muscles. The inoculation of virus into the trachea of rabbits does not lead to infection. The successful infection of rabbits has been attained by feeding virulent material with the food, and on occasion also by the introduction of virus into the stomach by means of a catheter passed down the œsophagus. On occasion, successful infection of the rabbit has followed the inoculation of the virus into the nasal mucous membrane. Attempts to infect rabbits by cohabitation have met with little success [vide Galloway and Nicolau (1929)].

Nature and Properties of the Virus.

Evidence of filterability. There is some evidence that the virus of Borna disease can pass, though with great difficulty, through bacteriological filters which hold back ordinary bacteria, and that the concentration of virus in the filtrate is much reduced. Zwick, Seifried and Witte (1926), carried out over 30 filtration experiments with different filters, and inoculated more than 100 rabbits with the various filtrates before they succeeded in infecting rabbits with them. Ernst and Hahn (1926, 1927), also recorded successful filtration of the virus. Nicolau and Galloway (1927 and 1928[1]), recorded two filtration experiments, Mandler filters being used, and they inoculated four rabbits with each of the two filtrates. That the concentration of the virus in the filtrates was greatly reduced was evidenced by the fact that in the first experiment, only one of the four rabbits fell ill from the disease and died, while in the second experiment, one out of the four rabbits was affected and showed only a transient paresis.

Effect of dilution and centrifugalization : adsorption. Few experiments have been recorded in which an accurate titration of the virus has been made : Zwick, Seifried and Witte recorded that the dilution of 1/10,000 of virulent brain is still capable of producing the disease. The writer has succeeded in infecting animals with a 1/20,000 dilution of brain. The centrifugalization of a virulent brain emulsion from which the larger particles had been removed, for 5 or even 15 minutes at 5,400 revolutions per minute does not deprive the superficial fluid of virulence (Nicolau and Galloway, 1927). The virus is not easily adsorbed by substances such as kaolin and animal charcoal (Nicolau and Galloway, 1929).

Effect of heat, desiccation and ultraviolet light. Zwick and his collaborators (1926) found that a brain emulsion heated at 57° C. in a water bath for 30 minutes, or at 70° C. for 10 minutes, lost its virulence, but when it was heated for 5, 10, 15, 20 and even 25 minutes at 50° C., the virulence was still preserved. In some cases heating for 30 minutes at 50° C. rendered a brain emulsion avirulent. Emulsions of brain retain their virulence for at least 306 days in the dry state (Nicolau and Galloway, 1929, 1930).

Nicolau and Galloway (1927) showed that the virus subjected to the action of ultraviolet rays emitted by a mercury arc (radiations of wavelengths 5,720—2,320 A.U.) is rendered non-infective in five minutes under the conditions of their experiment.

Action of glycerin, chloroform, ether, formalin and potassium permanganate. The virus of Borna disease is very resistant to glycerin. Zwick, Seifried and Witte (1927) found that Borna virus which had been 6 months in 50 per cent. glycerin was still fully virulent, but that a sojourn of 12 months in 50 per cent. glycerin had rendered it non-infective. Nicolau and Galloway (1928[1]) have shown that it may remain viable in glycerin in the cold room at 4° C. for at least 200 days. They are also of the opinion that rabbits infected intracerebrally with virus kept in glycerin succumbed to the infection at an earlier date than rabbits inoculated with an emulsion of fresh virulent brain. Levaditi, Harvier and Nicolau made a similar observation concerning herpes virus.

The virus is inactivated by chloroform, ether and formalin in a concentration of 0·2 per cent., after 18 hours contact at room temperature (Nicolau and Galloway, 1928[1]). Zwick, Seifried and Witte (1927) stated that Borna virus in a 4 per cent. brain emulsion with 1 per cent. phenol was still viable after 14 days, but not after 4 weeks. The virus does not appear to resemble that of vaccinia in its sensitiveness to potassium permanganate (Nicolau and Galloway, 1929).

Conservation of the virus. While virus emulsified in physiological saline becomes rapidly destroyed (virus inactive when tested on the fourteenth day), it can be preserved at least 30 days in ordinary tap-water at room temperature, or in milk at least 60 days, and possibly longer; in one experiment, active virus was still found in an emulsion of virulent brain in milk after 100 days. The supernatant fluid from a tube containing a portion of virulent brain in M/25 'buffered' phosphate at pH 7·5 was virulent after a sojoürn in the incubator of 48 hours at 37° C. (Nicolau and Galloway, 1928[4]).

Putrefaction rapidly inactivates the virus (5 days at room temperature) (Zwick, Seifried and Witte, 1927).

All attempts at cultivation of the virus made up to the present have been unsuccessful.

Distribution of the virus in organs and tissues of infected animals. Zwick, Seifried and Witte (1926) tested 4 samples of blood from infected rabbits, 3 samples of blood, 2 of spleen, 2 of kidney, and 2 of liver from horses ill of Borna disease, and failed to find the virus. Ernst and Hahn (1927), on the other hand, proved the blood to contain the virus during some stages of the illness of a rabbit suffering from Borna disease. Similar apparently contradictory results have been obtained in experimental infection produced by other filterable viruses. The presence of the virus has been demonstrated in the vitreous body of the eye (Ernst and Hahn, 1927), and in the submaxillary gland (Zwick and his collaborators, 1926). It has also been recovered from the cerebrum, medulla oblongata, spinal cord, parotid glands, adrenal glands and pancreas (Nicolau and Galloway, 1928[1]; Nicolau, S., Nicolau, O. D., and Galloway, 1928[2]). The presence of the virus has also been demonstrated by the latter authors in the peripheral nerves of rabbits and monkeys infected by the intracerebral route, in which infiltrative lesions in the nerve occur. Nicolau and Galloway (1928[1 & 2]) are of the opinion that the virus of Borna disease inoculated intracerebrally, travels centrifugally from the central nervous system towards the peripheral nervous system exclusively by nerve tracts, and thus behaves in a similar way to those of rabies, poliomyelitis, herpes, and probably also to some extent, neuro-vaccinia (Nicolau, S., Nicolau, O. D. and Galloway, 1929).

Infectivity of secretions. Zwick, Seifried and Witte (1926) and Ernst and Hahn (1927) have proved that the saliva and nasal secretions of infected animals are virulent, while the urine has always been shown to be avirulent.

Pathological Anatomy and Histo-pathology.

The lesions found in the central nervous system of cattle and sheep dead of enzootic encephalomyelitis are analogous to those produced by the virus of equine type. In general also it may be said that the tissue changes in the central and peripheral nervous system of large or small laboratory animals infected experimentally are analogous to those found in horses, cattle and sheep which have contracted the disease spontaneously.

Macroscopic lesions.

In the majority of cases, on naked-eye examination, congestion of the meninges is found, and this may sometimes be very intense; more rarely the aspect of the nervous system is normal.

Microscopic lesions.

Characteristic microscopic lesions of an intense nature are found only in the nervous system, and these may be classed in two categories, infiltrative and degenerative.

Lesions in the brain, cerebellum and medulla oblongata. The infiltrative lesions in the brain consist of meningeal infiltration, perivascular 'cuffing', and also parenchymatous infiltration. The cells taking part in the inflammatory process are lymphocytes, plasma and large mononuclear cells. A noticeable feature in the process of perivascular infiltration throughout the brain is the abundance of plasma cells, which are found in much larger number than in most other encephalites. The presence of polymorphonuclear leucocytes is exceptional, even in the earlier stages of infection. In the parenchyma itself, diffuse infiltration with lymphocytes accompanied by proliferation and mobilization of the neuroglial cells occurs, and is especially marked in the region of the cornu ammonis and around the lateral ventricles.

Zwick and his collaborators have repeatedly emphasized the fact that they have never found degenerative lesions of nerve-cells, nor neuronophagia in any region of the nervous system where they were searched for. More recently, however, Nicolau and Galloway (1927, 1928[1]), who have made a much more comprehensive study of the lesions throughout the nervous system of rabbits, monkeys, guinea-pigs, rats, and mice infected either with a strain of equine origin or one of ovine origin, have definitely shown that degenerative lesions of the nerve-cells and neuronophagia preceded by pathological ' satellitism ' can be found, not only in various parts of the brain and cord, but also in the spinal ganglia. Later, Dobberstein (1928) also has described neuronophagia and degenerative lesions in nerve-cells in the brain of horses affected with Borna disease.

Lesions in the spinal cord. Pathological changes are always found in the cord whether myelitic symptoms are present or not, but they are more intense when the symptoms produced by affection of that region dominate the cerebral symptoms. Only rarely is a severe meningitis found. It is similar to that occurring in the brain. Perivascular infiltration, together with small islands of lymphocytes unconnected with vessels, are found in both the white and grey matter. Frequently the zone of Lissauer is the site of a well-marked mononuclear infiltration. The anterior horn is the site more commonly of degenerative lesions of the neuron, while in the posterior horn the most intense infiltrative lesions are found. The types of degeneration in the nerve-cells are the same as in the other regions of the central nervous system.

Lesions in the peripheral nervous system. The spinal ganglia show very intense lesions. Between the nerve fibres which pass through the substance of the ganglion a well-marked mononuclear infiltration is seen. The capsule of the ganglion shows no infiltration; the small intra-ganglionic vessels show perivascular ' cuffing '. Infiltrating mononuclear cells are found disseminated between the nerve-cells or massed together, forming actual nodules, comparable with those described by Van Gehuchten and Nelis (1900) in rabies. The ganglion cells themselves appear to be choked by the infiltrative process. In certain parts these cells undergo profound changes: the nucleus becomes oxyphilic and the protoplasm loses its granular nature and also becomes slightly oxyphilic. Vacuolization of the protoplasm of the ganglion cells may be found. The most important and frequent type of lesion in the ganglion, however, is neuronophagia, which is commoner in the peripheral zone of the ganglion.

In the spontaneous disease in horses an inflammatory process in the optic nerve and in nerves in which paralysis is evident (N. facialis, hypoglossus, trigeminus) has been referred to by various authors. Interstitial neuritis has been demonstrated in the brachial and sciatic nerves including the nerve roots, pneumogastric nerve, and even in small cutaneous or intramuscular nerve filaments of animals infected by the intracerebral route. Lesions have also been described in intra-parenchymatous nerve-cells, ganglia and plexuses of the autonomic nervous system associated with various organs, for example, the heart, adrenal glands, pancreas, intestines, stomach, eye, parotid glands and lungs

(Nicolau, Nicolau and Galloway, 1929). This fact is of the greatest importance since it serves to show that the clinical symptoms in affected animals are not entirely central in origin. Sudden death which sometimes occurs in infected animals may be explained by the lesions produced by the virus in the autonomic nervous system of the heart. It is possible that intestinal stasis, respiratory distress, amaurosis, and digestive disturbances also may be the result in part of the lesions which occur in the peripheral nervous system.

Joest-Degen corpuscles. A noticeable feature in the histo-pathology of Borna disease is the presence of the Joest-Degen corpuscles (see Chapter II) within the nuclei of the nerve-cells. They may be found not only in the ganglion cells of the cornu ammonis, but also in the pyramidal cells of the cerebral cortex elsewhere, in the neuroglial cells (large granulo-adipose cells of the cornu ammonis), in the nerve-cells of the medulla oblongata and of the anterior horn of the spinal cord, in the spinal ganglia, and also in intra-parenchymatous nerve-cells, ganglia or plexuses, associated with the autonomic nervous system of internal organs. These intranuclear corpuscles are larger and in greater number in the spinal ganglia than in other sites, and are more easily found and more numerous in the monkey than in the rabbit infected experimentally. They may be single or in twos or threes, varying in size from the limit of visibility to 3μ or even 5μ or 6μ, and are generally surrounded by a halo. They stain a bright red with Mann's stain. An important point is that as a general rule only those cells maintaining a perfect morphological and tinctorial integrity contain the corpuscles of Joest-Degen and they are not found in the nuclei of nerve-cells which have undergone degeneration or are in a state of neuronophagia. The recent work of Nicolau, S., Galloway and Nicolau, O. D. (1928) has shown that the larger Joest-Degen corpuscles have a definite structure similar to that which has been described in Negri bodies in rabies.

Immunity.

Immunization of animals. Attempts to immunize the horse have been few in number. Zwick, Seifried and Witte (1927) found that three horses which had received several subcutaneous inoculations of virulent rabbit-brain at intervals, resisted a subsequent intracerebral inoculation of infective material, while two controls succumbed to the intracerebral inoculation. In the rabbit, experiments have been more numerous. Zwick, Seifried and Witte (1926) observed that in one case a rabbit which had been infected experimentally, and had shown symptoms typical of the disease, ultimately recovered and resisted a second intracerebral inoculation. They have succeeded on occasion in producing a solid immunity by repeated inoculations of the virus either subcutaneously or intravenously, and have shown that the introduction of a large quantity of virus intraperitoneally may render rabbits refractory to subsequent infection. Ernst and Hahn (1926 and 1927) recorded experiments which tended to show that inoculation of the virus intracerebrally into rabbits does not lead to the development of the disease if, during the period of incubation, such animals receive repeated inoculation of the same material into the veins. Nicolau and Galloway (1928[1]), made a similar observation on the guinea-pig. The susceptibility of the guinea-pig to virus inoculated intracerebrally appeared to be diminished by simultaneous inoculations of the same material intramuscularly. Zwick, Seifried and Witte (1927) failed in their attempts to repeat Ernst and Hahn's experiments. Nicolau and Galloway (1927 and 1928[1]) found that a rabbit which had received four intravenous inoculations of virulent emulsion at intervals became immune to a subsequent intracerebral inoculation, whereas rabbits which had received a lesser number of inoculations intravenously were not immune to the subsequent intracerebral inoculation. They also found to be immune to intracerebral inoculation one rabbit which had previously received infection by the intracorneal route without showing symptoms, and another rabbit which had resisted an intratesticular inoculation.

Zwick, Seifried and Witte (1927) found that virus which had been rendered innocuous by the action of carbolic acid, glycerin, heating to 50° C., or drying over caustic potash, was completely deprived of the property of immunizing the rabbit. Nicolau and Galloway (1928[1]) noted that such was the case also with virus inactivated by the action of ultraviolet light, ether, or chloroform. They also made some experiments, using virus inactivated by formalin as a vaccine; of 8 rabbits which received 5 doses of such a vaccine subcutaneously at 5 days' interval, only 3 resisted a subsequent intracerebral inoculation of the virus. More recently it has been shown that repeated inoculations of virus inactivated by formalin into the brain of animals may produce a certain degree of resistance but that the immunity is not absolute (Nicolau, Galloway and Stroian, 1929). They found that on one occasion a rabbit which had been inoculated intracerebrally with a diluted emulsion of virulent brain which had been pulped and preserved in glycerin at room temperature for several weeks, developed a slight paresis, but subsequently recovered and resisted a subsequent intracerebral inoculation of fresh virus 54 days after the first infection. It would appear that some living virus was still present in the material preserved in glycerin at room temperature which produced a mild attack of the disease resulting in immunity.

Virus which has been treated with carbo-glycerin (carbolic acid, 0·5 gm.; distilled water, 39·5 gm.; glycerin, 60 c.cm.) for 7 to 9 days at 24 to 26° C. possesses good immunizing properties (Nicolau, Galloway and Stroian, 1929).

Cross immunity between the strain of equine and that of ovine and bovine origin. Rabbits which have become resistant to infection with the virus of encephalomyelitis of equine origin prove also to be refractory to infection by intracerebral inoculation with the virus of ovine origin, while the converse is also true (Nicolau and Galloway, 1927). It has also been proved that the equine strain cross-immunizes with the bovine strain (Nicolau and Galloway, 1929, 1930).

Cross immunity between the virus of enzootic encephalomyelitis and other viruses of the filter-passing group. Animals immune to herpes and poliomyelitis are still susceptible to the virus of Borna disease (Nicolau and Galloway, 1927, 1928[1]; Nicolau, Galloway and Stroian, 1929). Rabbits immune to Borna disease are susceptible to an intracerebral inoculation with the 'street' virus of rabies (Nicolau and Galloway, 1928[1]).

Occasional prolonged latency of the virus in susceptible animals. The virus of Borna disease can remain latent for long periods in the organism of susceptible animals. Although in passaging a strain adapted to rabbits no animal is ever found to be immune to intracerebral inoculation with a thick, virulent emulsion, exceptionally long incubation periods do occur. Nicolau and Galloway (1928[1]) recorded a case in which a rabbit which had been inoculated with a brain emulsion apparently containing very little virus developed a recurrent form of the disease to which it succumbed 161 days after the inoculation. On occasion also, longer periods of incubation may been countered, as, for example, in one case (unpublished observation) these authors found that a rabbit inoculated with a 10 per cent. emulsion of virulent brain did not succumb to the infection until the one hundred and seventy-third day. For this reason one must not be too hasty in concluding that absolute immunity exists. They also here record that a guinea-pig was inoculated intracerebrally with a virus of monkey passage, showed no symptom during 350 days, and then developed paresis and died 373 days after the inoculation. Typical lesions of Borna disease were found throughout its nervous system, and rabbits infected with emulsions made from the brain and cord succumbed to Borna disease. The temperature of the environment of this guinea-pig had been considerably lowered some short time prior to the development of the first symptoms, and it is possible that the altered conditions under which the animal was placed were sufficient to light up a dormant infection. The experiment recorded in their monograph (1928[1]), where the inoculation of the virus of poliomyelitis into a monkey which had resisted for a long period the virus of Borna disease provoked the dormant infection with the latter virus, is also suggestive. The experiment has an added interest because from the brain of the monkey, which subsequently died, the virus of Borna disease was recovered while that of poliomyelitis was not. Furthermore, another observation may be cited pointing in the same direction. Attempts at infecting rabbits by cohabitation made by Zwick, Seifried and Witte (1926, 1927), and Nicolau and Galloway (1927, 1928[1]), have given negative results. However, Galloway and Nicolau (1929) kept normal rabbits with infected rabbits for a long period and subsequently inoculated them intracerebrally with physiological saline. One rabbit of this group developed Borna disease as proved by passage and histological examination of its nervous system. It would appear that the irritation of the brain had lit up a latent infection. The suggestion that the virus of Borna disease may remain latent in the system and be reawakened recalls to our minds the observation of Pasteur, that certain rabbits which were resistant to the inoculation of an attenuated rabies virus at once showed paralytic symptoms after exposure to severe cold.

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CHAPTER XXIX. LEUKÆMIA OF FOWLS.

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Clinical Features.

THIS disease of the domestic fowl is of special interest because of the resemblance which it bears to leukæmia of man and also because it is one of the few examples of a disease of the blood which is capable of experimental transmission. It occurs not infrequently in this country and on the continent, and cases have been observed in America. Although never giving rise to extensive epidemics, the occurrence of a number of cases together on the same farm is not uncommon, and is suggestive of the contagious nature of fowl leukæmia. Ellermann (1921[1]), who has made an extensive study of this disease in Denmark, recognizes three clinical types: the lymphatic, the myeloid and the intravascular lymphoid or anæmic.

The lymphatic type. In this type of disease there appear to be few or no clinical symptoms. Occasionally some anæmia, as evidenced by paleness of the comb, may develop prior to death, but as a rule death occurs unexpectedly, and the nature of the disease is only revealed by the post-mortem examination. The blood picture is normal. The liver is enlarged up to eight times its normal size and mottled in appearance. The spleen is enlarged. The kidney may show a similar mottling to that seen in the liver and the bone-marrow, though usually normal in appearance, and may contain a number of small whitish nodules. Microscopically the underlying feature of this condition is seen to be a hyperplasia of the lymphoid tissue wherever it normally occurs (spleen, liver, intestine, kidney, skin and bone-marrow). It is the hyperplastic areas which are responsible for the mottled appearance. The majority of cells present in this lymphoid tissue are lymphoblasts.

Myeloid type. Birds suffering from this type of the disease present symptoms recognizable during life. They appear ill, their combs are pale and they are listless and off their feed. The illness is of short duration, death ensuing in the majority of cases after a course of some ten days. In contradistinction to that of the lymphatic type the blood picture in myeloid leukæmia is definitely pathological, although aleukæmic cases do occur. There is a certain degree of anæmia, the number of erythrocytes being diminished and the hæmoglobin percentage reduced. The outstanding feature of the blood picture, however, is the enormous increase in the number of leucocytes. Counts of from 200,000 to 600,000 per c.mm.

are met with, the normal white count in the hen being in the neighbourhood of 30,000 per c.mm. Ellermann (1921[1]) gives the following differential count as characteristic of this type of the disease:

Polymorphonuclears	2 per cent.
Myelocytes	21 ,,
Large mononuclears and poikilonuclears ..	67 ,,
Lymphocytes	5 ,,
Lymphoidocytes (primitive undifferentiated cells)	5 ,,

The name poikilonuclear is given by Ellermann to large pathological cells devoid of granules but possessing a more or less polymorphous nucleus. They obviously represent some abnormal developmental stage of the polymorphonuclear.

The normal differential leucocyte count in the hen is:

Polymorphonuclears	33 per cent.
Mast cells	2 ,,
Mononuclears	12 ,,
Lymphocytes	53 ,,

Myeloid leukæmia of the hen is almost invariably fatal, and on post-mortem examination the liver and spleen are found to be moderately enlarged. Microscopic examination of the organs reveals a myeloid hyperplasia in the liver, kidney and spleen, this change being most prominent in the liver, where huge accumulations of myeloid cells, principally myelocytes, are found in the periportal areas. The bone-marrow shows little or no hyperplasia of myeloid tissue. In addition to these changes there is an accumulation of abnormal blood-cells—myelocytes and primitive undifferentiated cells—in the capillaries of the liver, kidney and bone-marrow and in the vascular spaces of the spleen. The condition has been termed leucostasis by Ellermann.

Anæmic type (*Erythroleucosis, intravascular lymphoid leucosis*). The clinical picture in this type of the disease is more definite than in the preceding ones. The bird is quiet and does not feed, but the outstanding feature is the yellow colour of the comb and other visible epidermal structures. Ellermann briefly refers to the yellow colour of the comb, and Schmeisser (1915) erroneously described the condition as jaundice. Bedson and Knight (1924) showed that this pigmentation was not due to bile-pigment, but to the normal lipochrome of the hen, xanthophyll, which is derived from the food. This lipochrome is taken up by the ether-extractable substances (fats and lipoids) present in the plasma, and deposited in various epidermal structures, such as the comb, wattles, eye ring, beak and leg scales, as well as in the egg yolk and the body fat generally. The striking yellow colour of the hens is therefore merely an exaggeration of a normal process, and the underlying pathological condition is an increase in the fatty substances in the plasma, the vehicle of the lipochrome (Bedson and Knight, 1924).

The total blood count is low, one million or less, as against the normal figure of three million. Bedson and Knight record total counts as low as 408,000. This reduction is entirely at the expense of the erythrocytes. The leucocytes are generally normal in appearance and number, though some myelocytes may be present. There are, however, a large number of abnormal cells of the erythrocyte series—polychrome erythrocytes, erythroblasts and primitive undifferentiated cells (lymphoidocytes)—present in the circulation.

Pronounced variations in the degree of anæmia are met with, and the course run by the disease is variable. Remissions of shorter or longer duration occur not uncommonly (Ellermann, 1921[1]; Bedson and Knight, 1924); apparently in the lymphoid and myeloid types of the disease this is not the case. Whether this type of fowl leukæmia invariably terminates fatally is not known, though the probability is that the majority of affected birds succumb either to the first attack or one of the subsequent relapses. The post-mortem findings consist of a yellow pigmentation of the skin, more particularly that of the comb and around the oil gland. The body fat may be generally increased and of a more intense yellow colour than normal. The liver is enlarged and paler and yellower in colour than normal. The spleen is enlarged up to four or five times its normal size. Microscopically the characteristic finding is an accumulation of cells representing the various developmental stages of the erythrocyte, in the capillaries of the bone-marrow, kidney and liver, and in the vascular spaces of the spleen pulp. In the bone-marrow the myeloid tissue may show a considerable reduction, and the follicles of the spleen are frequently small or quite atrophied. Large endothelial phagocytes packed with yellow pigment and cell debris are to be seen in the spleen and liver. In addition to the pure anæmic type of the disease, mixed forms occur in which both the erythrogenic and myeloid tissues are implicated—an erythroleucosis or erythromyelosis (Ellermann).

Susceptible Animal Species.

The hen would appear to be the only susceptible animal. Ellermann and Bang (1908) inoculated rabbits, guinea-pigs, doves, guinea-fowl and turkeys with material from cases of fowl leukæmia and obtained negative results. Similar negative findings in attempts at heterologous transmission have been recorded by Hirschfeld and Jacoby (1909–10) (rabbits, guinea-pigs and doves), and by Bedson and Knight (1924) (guinea-pigs and pigeons).

Ætiology.

The view that this disease is due to an ultramicroscopic organism depends on the following facts:

1. The inability to demonstrate a bacterial cause by either cultural or microscopic means.
2. The ability to transmit the disease in series with material free from visible or cultivable organisms.

3. The possibility of transmitting the disease with bacteria-free and cell-free filtrates of virulent material.

It will be necessary to examine this evidence in greater detail.

Inability to demonstrate a causal bacterium. Although Moore claimed to have isolated an organism which was capable of reproducing the disease, and Burckhardt (1912) considered that the condition was due to infection with an avian tubercle bacillus of low virulence, subsequent work goes to disprove these views. Bacterial infection in the hen may bring about extensive changes in the blood picture and tuberculosis in the fowl may be associated with a definite anæmia and a leucocytosis which sometimes assumes enormous proportions. Recent work on this disease has, however, revealed no constant bacterial findings and the filtration experiments of Ellermann and Bang (1909) and Ellermann (1913) definitely point to an ultramicroscopic virus as the cause of fowl leukæmia.

Ability to transmit the disease experimentally. This possibility was originally demonstrated by Ellermann and Bang (1908). They showed that an emulsion of organs (liver, spleen and bone-marrow) from spontaneous cases when injected intravenously in healthy birds reproduced the disease. Their transmission experiments were by no means invariably successful, for, out of the 83 birds inoculated by them, only 34 developed the disease. The incubation period was a long one, some three to four months. Their work has been confirmed (Hirschfeld and Jacoby, 1909–10; Ellermann, 1921[2]; Schmeisser, 1915), though here again only a low percentage of transmissions was obtained. The experiments of Bedson and Knight (1924) were less satisfactory, for only a transitory condition was produced in 3 out of the 13 hens inoculated by them. It might be objected to in these transmission experiments that the experimental animal is one in which the disease occurs spontaneously, and there is no evidence that any attempt was made to obtain a clean experimental stock; but it is quite obvious that even the lowest percentage of positive results recorded by Ellermann, viz. 22 per cent. (17 out of 79 birds inoculated), represents an incidence very much higher than that which occurs naturally. Were this not so, fowl leukæmia would be a much more serious problem on hen farms than it is. Nevertheless, the possibility of the inoculation lighting up a latent infection should not be overlooked, and future work should be carried out with hens reared under conditions obviating the possibility of outside infection. Ellermann has succeeded in realizing twelve passages with one of the strains with which he worked, and this fact together with the progressive shortening of the course of the disease in the succeeding generations which he has observed, strongly supports the contention that the disease is due to a living agent.

Evidence has been produced to show that all three types of fowl leukæmia are caused by the same agent (Ellermann, 1921[2]). This point is well brought out in the experiments carried out with a strain of virus obtained from a spontaneous case of the erythroleucotic type. In the first passage this virus produced a myeloid leukæmia, whereas in the second generation it gave rise to both an erythroleucosis and lymphatic leukæmia. What the factors are which determine the type of disease produced is not known, nor have any observations been made which throw any light on the mode of spread of fowl leukæmia in the field. These are both important points which require investigation, though work with this virus is rendered difficult by the relatively small percentage of birds which can be infected experimentally. As Ellermann has pointed out, at least ten birds should be inoculated in each experiment to ensure that at least one bird contracts the disease, and this entails working on a very large scale. It is somewhat surprising that with the increase in virulence brought about by passage of the virus, the number of takes is not also increased. This observation of Ellermann suggests that negative results can hardly be explained by postulating an immune state, for one would not expect to find absolute immunity or its complete absence without any intervening stages, unless one were dealing with a natural immunity possessed by certain breeds of hen. Further work is required to elucidate this point.

Filterability of the Virus.

The filterability of the virus of fowl leukæmia rests on evidence produced by Ellermann and Bang (1909) and Ellermann (1913). They made use of emulsions of organs in saline which were clarified by a short centrifugation (2,000 to 4,000 r.p.m.). Six experiments were performed with Berkefeld filters and of these five gave positive results. Altogether 42 birds were inoculated with bacteria-free filtrates in these six experiments, the dose used varying from 1·0 to 10·0 c.cm. inoculated intravenously. Of these 42 birds, 8 developed the disease, a percentage a little lower than that obtaining in experiments with unfiltered material. Ellermann's experiments with Reichel (porcelain) filters proved less successful, only one filtrate out of three being active. This positive result is of additional interest, however, for the hen from which the material came was suffering from tuberculosis as well as leukæmia. Two of the ten birds inoculated with the filtrate developed leukæmia, none developed tuberculosis. The interpretation of these findings is open to the same criticism as has already been made of the transmission experiments, viz. that the experimental animal is one which contracts the disease spontaneously. In future confirmatory work this possible source of error should be controlled.

Immunity.

There is a complete absence of information on this aspect of the disease. Reference has been made to the fact that the domestic fowl alone would appear susceptible to this virus. Whether the different breeds of hen are equally susceptible requires investigation.

Possible Relationship between Leukæmia of Fowls and the Leukæmias of Man and Other Animals.

The temptation to liken this disease of hens to the leukæmias of other animals, especially of man, is very great, and although it has to be admitted that there are superficial resemblances, a close examination of the facts reveals fundamental differences. Foremost amongst these is the possibility of transmitting fowl leukæmia experimentally, for in none of the other leukæmias has this been achieved. Then again the virus of fowl leukæmia would appear capable of evoking indifferently a myeloid leukæmia, an aleukæmic lymphatic leukæmia and a megaloblastic anæmia, whereas in man there is no evidence that leukæmia is in any way connected ætiologically with pernicious anæmia. The specific response of human pernicious anæmia to liver treatment is additional evidence on this point. It would appear more reasonable to look upon fowl leukæmia as an infectious disease peculiar to the hen, akin to the Rous sarcoma, and to read into human pathology the findings obtained from either of these diseases of hens appears unwarranted.

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CHAPTER XXX. INFECTIOUS ANÆMIA OF HORSES.

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Synonyms. Anémie infectieuse : Infektiöse Blutarmut der Pferde : Ansteckende Blutarmut der Pferde : anæmia infectiosa equorum : swamp fever of horses : equine pernicious anæmia : malarial fever of the horse : anémie pernicieuse : typho-anémie infectieuse du cheval (Carré and Vallée) : anémie epizootique.

Description of the Disease.

Infectious anæmia is a specific infectious disease of the horse, characterized by an acute or chronic febrile septicæmia with a progressive diminution in the number of erythrocytes and a varying degree of emaciation. It is produced by a filterable and ultramicroscopic virus. The affected animal does not always appear anæmic.

Clinical Symptoms.

The period of incubation after artificial infection is very variable. Carré and Vallée (1904, 1906, 1907) give 5 to 9 days. Nagao (1923) found it to be 9 to 22 days in 70 per cent. of cases, but symptoms may not develop till many months after infection.

Two clinical forms can be recognized, the acute type and the chronic type. In acute cases the onset is rapid. The horse shows great weakness, loss of appetite, and there is a gradual rise in temperature up to 40° C., or even 42° C. The conjunctival and nasal mucous membranes have a peculiar diffuse red coloration. A bloody diarrhœa may be present; there is albumin in the urine and a rapid emaciation results. Cardiac disturbances are evident. Death follows in from 5 to 15 days. In the acute type indications of anæmia are absent, and the modifications of the blood are not marked. The coagulation time is lengthened. In subacute cases the symptoms are less intense and there are apparent remissions; death may occur in a few weeks to some months.

The chronic type is characterized by periodical febrile attacks and signs of anæmia. Such cases may last for many months. The erythrocyte count may fall from the normal of about 7,000,000 to 2,000,000 per c.mm. or even lower, the diminution being in proportion to the duration of the disease. There is a slight hypoleucocytosis in chronic cases, while in acute cases there may be a polymorphonuclear leucocytosis, but the blood changes affect chiefly the red blood-cells.

The lesions at autopsy vary according to the evolution of the disease and are found chiefly in the heart, spleen, lymph glands and bone-marrow, while accessory lesions may be present in the kidney and liver.

Epizootiology.

The disease was first recognized by Lignée (1843) in France. Delafond (1851) attempted its transmission from affected to healthy horses and to sheep without success, and it was Anginiard (1859) who first suggested that it was contagious. It has been reported in various parts of France, Italy, Austria, Japan, Russia, South Africa, Morocco and America, and was recorded during the War on the different fronts. During 1918 in the German army, there were nearly 3,000 horses affected in 89 different centres. As a result of demobilization, new centres of infection sprang up after the War. The disease causes great losses.

Ætiology.

Vallée and Carré (1904, 1906, 1907; Carré and Vallée, 1904) first transmitted the disease from affected to healthy horses by inoculation of blood. They also proved that the virus can pass a Berkefeld V or a Chamberland B or F candle, although the latter holds back a considerable proportion. These observations have been amply confirmed by the Japanese Commission (1914) and others.

Miyagava, Taniguchi, Nagao and Takemoto (1918) claim to have cultivated an organism which they call *Spirochæta equi infectiosa* from a case of infectious anæmia. Their findings have not been confirmed.

While Seyderhelm (1918) attributed the disease to the toxic action of *Gastrophilus* larvæ, de Kock (1919) has shown that although these larvæ may carry the virus, their toxin is not the cause of the disease.

Modes of Infection.

The disease can be reproduced experimentally by subcutaneous or intravenous inoculation of small quantities (0·01 c.cm.) of blood, plasma or serum. The result of the inoculation is not controlled by the size of the dose of virus administered ; small doses may produce an acute form, while large doses of 700 c.cm. of infective blood may produce a chronic form. Horses can be infected by the feeding of relatively large doses of virulent material. Under natural conditions ingestion is probably a common method of infection. The Japanese Commission (1914) suggested the possibility of transmission by biting flies such as Tabanidæ, *Stomoxys* and *Simulium ;* this possibility has been further studied by Scott (1920) ; while the results of their experiments are not conclusive, they have given support to the suggestion, since Scott showed that the simple introduction under the skin of a needle charged with virus is sufficient to produce infection.

Distribution of the Virus in Various Organs and Tissues : Infectivity of Secretions.

The virus can be found in the blood and all the organs, including the spinal cord and bone-marrow. It is eliminated in the urine and the milk. The infectivity of the conjunctival and nasal secretions and saliva has been suggested, but not conclusively proved.

Animals Susceptible.

All breeds and ages of horses are susceptible. The donkey has been infected (Vallée and Carré, 1906, 1907; Japanese Commission, 1914). The Japanese Commission inoculated two young pigs with 10 c.cm. of virulent blood. Both died within 5 days after a febrile reaction, but it was not proved that they had died of the inoculation. However, the Commission took blood from a half-grown pig which had been inoculated at the same time and had shown no symptoms, one month afterwards, and with it reproduced the disease in the horse. Habersang (1919) found that the virus remained viable in the pig's body for 38 days. Lührs (1920^1) found that the blood of pigs inoculated with the virus could remain virulent for the horse for 143 days. As a result of the inoculation pigs may show febrile symptoms and a fall in the number of erythrocytes, but little importance can be attached to the latter symptom.

Attempts to infect cattle, dogs, monkeys, cats, rats, mice, hedgehogs, sparrows and pigeons have given negative results, while inconclusive results have been obtained with hens, sheep and goats. It is interesting to observe that Donatien and Lestoquard (1924) have described an infectious anæmia among sheep and goats produced by a filter-passer which could be transmitted to the ass and to calves.

Jaffé and Silberstein (1922) inoculated rabbits with material from infected horses, and observed symptoms and blood-changes which they attributed to the action of the virus, but they could not reinfect horses with blood from the rabbits. Helm (1924, 1926), Neuwerth (1924), Schulze-Gahmen (1924), and others have had similar results in attempts to infect guinea-pigs. Oppermann and Lauterbach (1923), Oppermann (1924) and Ziegler and Grosse (1925) have advocated the use of the rabbit for experimental and diagnostic purposes; but Schermer and Eigendorf (1926) have produced anæmia with emaciation and febrile reaction in rabbits with normal horse- or pig-serum or extracts of gastrus larvæ, and have shown that 15 to 20 c.cm. of blood from a rabbit showing an anæmia so produced will reproduce the condition in a sound rabbit. It is thus clear that the development of anæmia in the inoculated animal must not be taken as the sole criterion of successful transmission even in the horse. In one experiment of the Japanese Commission the erythrocyte count fell from 8,000,000 to 2,700,000 in 24 hours on account of isolysin present in horses' blood.

The great objection to the rabbit experiments is that the symptoms and blood changes produced have never been proved to be due to the specific action of the virus by passage back to the horse.

Transmission to Man.

Lührs (1920^2), who had been working with cases of infectious anæmia during the war, and had eaten the flesh of an infected horse, and been accidentally pricked with an inoculation needle, developed febrile symptoms, diarrhœa, pain in the kidneys and signs of anæmia. His blood for three years produced symptoms of infectious anæmia on inoculation into horses. Later, Peters (1924) described a case of severe anæmia in a patient who had been working with horses suffering from infectious anæmia. The clinical symptoms and blood-picture suggested an infection with the virus from horses; 1 c.cm. of the blood of this patient, filtered or unfiltered, produced in healthy horses all the symptoms of infectious anæmia followed by death.

These observations in man, if confirmed, and those of the Japanese Commission (1914), and Lührs (1920[1]), on the survival of virus in pigs are of particular interest. Horses are used for the production of immune serum against swine erysipelas, and if a horse with infectious anæmia were employed, there is danger not only of fatal symptoms being produced in pigs, but also of the inoculated pigs becoming a reservoir of the virus. It must also be remembered that horses are used for the production of antisera for human injections. That infectious anæmia virus may contaminate sera has been proved by de Kock (1920) in observations on the immunization of horses against horse sickness.

Nature and Properties of the Virus.

The virus is ultramicroscopic, and is capable of passing through filters which hold back bacteria. It has not been cultivated outside the animal body.

Effect of heat and cold. Heating to 56° C. for 60 minutes does not destroy the virus. Vallée and Carré (1907) found that heating to 58° C. for 1 hour kills it, but Hempel (1909) did not inactivate it by heating for a similar period. The Japanese Commission (1914) found that heating for 1 hour at 60° C. killed it. The virus will keep at —9° C. for at least 1 month (Japanese Commission) and according to Carré and Vallée, under 0° C. for 6 months. Nagao (1923) found that blood remained infective when suspended in citrate saline in the proportion of 3 : 1 for 1 year at —2° C.

Effect of desiccation. The virus will keep for some time in the dry state; Carré and Vallée (1906, 1907) say 10 days, but not one month.

Effect of disinfectants. Heiermann (1923) found that 0·5 per cent. carbolic acid kills the virus in 4 to 6 weeks and 0·1 per cent. after 8 weeks. The Japanese Commission found that 2 per cent. carbolic acid killed it in 1 hour; 3 per cent. cresol water does not kill it in 24 hours.

Immunity and Virus Carriers.

It is doubtful whether horses which have passed through an attack of the disease ever absolutely recover. In some cases there may be some lasting improvement, but a relapse may occur at any time. Carré and Vallée (1904) were the first to observe that horses which appear to have absolutely recovered from the chronic form of the disease still preserve the virus in their bodies and are consequently capable of infecting other susceptible horses. Schalk and Roderick (1923) kept a horse which had been inoculated experimentally with the virus and had not shown signs of anæmia for 14 years. During this period the animal was bled on 18 different occasions and the virus recovered from its blood infected fresh horses. Ultimately the animal showed acute anæmia without any obvious provoking cause.

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CHAPTER XXXI. AFRICAN HORSE SICKNESS.

BY SIR ARNOLD THEILER, K.C.M.G.

Definition and Nomenclature.

HORSE SICKNESS, a disease of equines, caused by a filterable virus, occurs mainly in Africa. It appears sporadically and enzootically in topographically well-defined areas. It may become epizootic in character and may spread over large areas. Wet years and humid localities favour the disease. The morbidity is greatest in horses and mules; asses are practically immune. The mortality is almost as great as the morbidity, especially among horses. The majority of the animals which recover are immune.

In the British possessions in Africa, the disease is known under the name of Horse Sickness. The present Afrikaans equivalent is 'Perdesiekte'; the German name is 'Pferdesterbe'. In French it is known as 'La peste du cheval', and in Italian, 'La peste equina'. According to certain symptoms the disease is often called Dunkop (siekte), Dikkop (siekte) or Blauwtong (Bluetongue) in South Africa.

History and Geographical Distribution.

The earliest historical references to horse sickness are found in an Arabian document 'Le Kitâb El-Akouâl El-Kâfiah Wa El Chafiâh' (L. Moulé, 1896). An outbreak occurred in Yemen in the year 728 of the Hegira (1327–8).

A later account is that of Father Monclaro, who described a journey of Francisco Baro in 1569 to the Zambesi, where horses brought from India died showing symptoms of horse sickness.

The disease is mentioned in many of the documents of the Dutch East India Company. Seventeen hundred horses died of it in 1769, and great losses are reported in 1780, 1801 and 1819. The greatest outbreak on record is probably that of 1854, when about 70,000, or over 40 per cent. of a horse population of 160,784 perished. The disease is now almost unknown in the Western and Southern Cape, and it is very rare in the arid North-Western Districts. It is found in parts of the Orange Free State, in the Transvaal, in Natal, in Southern and Northern Rhodesia, in the South-West Mandated Territory and in Portuguese East Africa. It is present in the whole of the eastern and central portion of the African continent, reaching the Soudan and the Red Sea in North Africa. It is not so well known in Northern West Africa, and is said not to occur in the Cameroons and Nigeria.

Horse sickness was at first not recognized as a specific disease. Lambert (1881) in Natal and Sander (1895) in German South-West Africa considered it to be identical with anthrax. Nunn (1888), Rickmann (1900) and Kuhn (1911) related it to human malaria. Edington (1895) at one time thought it to be caused by a fungus and called the disease *Œdema mycosis.* M'Fadyean (1900), Nocard (1901) and Theiler (1901) were able to demonstrate that the infective material is filterable.

Epizootiology.

Horse sickness is only found in certain localities and under certain orographic, telluric and climatic conditions. During the late summer months, after the rains, it occurs regularly with more or less severity in enzootic form in the low-lying coast lands, valleys and swamps. The appearance of the disease and the severity of the outbreak are governed by the amount and distribution of the rainfall. When the rains appear late, horse sickness also appears late. Enzootics usually terminate with the first frosts. Continuous rains do not favour the spread of the disease, but heavy rains alternating with hot spells, or followed by drought, furnish optimal conditions for its dissemination. In South Africa, March and April constitute the usual horse-sickness season for the middle and high veld, whereas in low-lying lands it may occur during May and June, and in years with very late rains may be found throughout the winter.

It has long been known that stabling animals at night and allowing them to graze only by day will prevent infection. Theiler (1903) and Pitchford (1903) expressed the opinion that horse sickness is insect borne, and they proved that animals housed in mosquito-proof stables are absolutely protected.

Ætiology.

Horse sickness is not directly contagious. It is, however, easily inoculable. The virus is present in blood, in serum, and in the serous exudates often found in the lungs, pleural cavity, and pericardium. It may be found in the blood from the first day of the fever until a few days after recovery. Under special circumstances, after transfusion of large quantities, blood has been shown to be still infective after 96 days. Liver and brain tissue washed free of blood is not infective (Theiler, 1901). Repeated washing does not free the red corpuscles from the virus, nor does continued centrifugation or long standing (three months) precipitate it from the serum (Sieber, 1911). Peritoneal exudate obtained by injecting peptone bouillon into the peritoneal cavity becomes infective. The virus may be transmitted by intrajugular and by subcutaneous injection. Large quantities given intratracheally and *per os* (minimum 150 c.cm.) are infective. The blood of a fœtus of a mare dead of horse sickness was found to be infective, likewise the milk of a mare that foaled during a horse-sickness reaction. In the latter case the minimum infective dose was found to be 0·0001 c.cm. The infectivity of a given virus depends to a great extent on the particular strain and on an apparent natural resistance of the animal injected.

Filterability of the Virus.

Microscopical examination of the blood and the usual methods of cultivation on artificial media have failed to demonstrate the growth of organisms. Positive filtering experiments were performed by M'Fadyean (1900), Nocard (1901), Theiler (1901) and Sieber (1911). The latter found that the virus can pass through the Berkefeld and the Chamberland F and B filters. He also found that the virus (serum) sufficiently diluted (2 per cent.) will pass through the Berkefeld, Pukall and Chamberland F filters, but is retained by the Chamberland B filter. In a dilution of 33 per cent. it is retained by the Chamberland F filter. It will not pass through a candle coated with 1 per cent. agar or collodion, or through one soaked in the latter.

Resistance of the Virus.

The resistance of a virus appears to depend on the initial virulence. Undiluted virus or blood diluted with glycerin may still be infective after four years. Less virulent viruses may lose their virulence in a year; 5, 10 or 20 c.cm. of such a virus may no longer be virulent, but 50 c.cm. may produce typical fatal horse sickness, and it must be assumed that the organisms have undergone a numerical reduction and not an attenuation. Putrefaction does not always destroy the virus, but it is destroyed by drying and by heating to 70° C. for 5 minutes, or to 50° C. for 10 minutes, though not by heating to 45° C. for 6 days. Sodium taurocholate (5 to 10 per cent.) and saponin do not destroy the virus (Sieber, 1911). Lecithin (5 to 10 per cent.) renders it avirulent, but the virulence is regained after separation of the lecithin. Ox-bile does not affect the virus, but Kuhn (1911) states that attenuation occurs when a mixture of the virus and dog-bile is allowed to stand for 24 hours.

The preservative mixture used at Onderste Poort consists of 500 c.cm. water, 500 c.cm. glycerin, 5 gm. sodium oxalate and 5 gm. carbolic acid. To a given quantity of infective fresh blood is added an equal amount of this mixture.

Susceptible Animals.

Horses are most susceptible to horse sickness; mules are less susceptible. Asses are reported to have died of the disease in bad horse-sickness years, but the author has never seen an ass succumb to an injection of virus. Zebras (Rickmann, 1908) and zebroids (Lichtenheld, 1907–8) are reported to have died of the disease. Some horses and mules resist experimental infection.

The mortality may reach 90 to 95 per cent. in horses and is slightly lower in mules. Of 54 dogs infected (Theiler, 1907, 1910), 30 developed typical fever reactions, and 9 died with typical lesions (pulmonary form). Dogs may contract the disease when fed on the flesh or organs of diseased horses (Bevan, 1911). Inoculation of the virus into young Angora goats results in a fever reaction (Theiler, 1910). Virus obtained from these goats produced fever reactions in dogs, but not in horses, but the virus from the dogs was virulent for horses. The virus lost its virulence for goats after a few passages.

In the writer's experiments, cattle did not react to the horse-sickness virus. Edington (1904), and recently van Saceghem (1918), have reported positive results, and state the disease is identical with heartwater of ruminants. But, according to Cowdry (1925), heartwater is caused by a *Rickettsia*, and, therefore, cannot be identical with horse sickness.

Susceptibility to horse sickness is a relative conception. Certain horses and mules may resist infection with small doses, and react later to larger doses of the same virus, or, they may resist a subcutaneous, and react to a subsequent intrajugular injection. It is believed that young horses are more susceptible than old. Foals of immune mares are said to be less susceptible or even immune. In one of the writer's experiments, 6 foals ranging in age from a suckling to 20 months and 22 days, born of mares immunized before the beginning of pregnancy, reacted to an inoculation of the virus, and 2 died. A foal born while its dam was undergoing a horse-sickness reaction succumbed to horse sickness when tested at the age of 5 months and 6 days. Two foals of mares which underwent a horse-sickness reaction and a subsequent test with another virus, gave atypical reactions when tested with virus. The blood of these two foals failed to infect horses which proved susceptible to a later virus injection. These experiments indicate that neither foals of immune mares, nor foals born during or shortly after the dam's recovery from horse sickness, acquire any immunity. In one case a foal born while its mother was undergoing a reaction died of horse sickness. Both the milk of the mother and the blood of the foal produced horse sickness in susceptible animals. Foals that were *in utero* at the time the mare was suffering from the disease appeared to have acquired immunity.

Natural Transmission.

Horse sickness is not directly contagious. The restriction of its occurrence to definite localities under definite atmospheric conditions suggests that transmission is brought about by a living vector, probably a nocturnal insect. Early recorders observed a similarity between the epidemiology of malaria and the epizootiology of horse sickness and some considered the two diseases identical. Horse sickness may occur wherever malaria occurs, but malaria does not occur wherever horse sickness occurs. This excludes mosquitoes of the genus *Anopheles* as vectors, despite Pitchford's (1903) apparently positive experiments. Schuberg and Kuhn (1912) successfully transmitted the disease with *Stomoxys calcitrans*, transmission in this case being mechanical. Williams (1913) made *Lyperosia minuta* responsible for an outbreak in the Soudan, and van Saceghem (1918) a *Tabanus* and a *Culicoides* species, in the Belgian Congo. These insects may come under consideration as mechanical carriers but not as intermediate hosts. Ticks also cannot be regarded as transmitting agents.

The question of the existence of a virus reservoir must be considered in connection with natural transmission, to account for the preservation of the virus during the period of about nine months, lasting from the first frosts till late the following summer, in which horse sickness does not occur, and horses may run on the veld day and night without danger of infection. It has been suggested that zebras may act as virus reservoirs, but against this is the fact that horse sickness may occur when susceptible animals are introduced into areas where there are no zebras or other equines. Possibly, as in trypanosomiasis, the virus may be harboured in game or other animals for which it is not pathogenic, but the widespread occurrence of horse sickness in severe epizootics in localities where game is scarce or absent restricts this possibility to small mammals, birds, reptiles or amphibians. The failure to produce horse sickness by injecting the blood of such animals into susceptible horses does not, however, prove that there is no virus reservoir (Theiler, 1912).

Taking into consideration all the facts, the writer is inclined to think that a virus reservoir (analogous to that found in trypanosomiasis) is not essential for the maintenance of the virus. To explain the epizootic occurrence of the disease on high plateaus and in arid areas, such as the Karroo, which is free of swamps and rivers, we must assume an insect carrier breeding in stagnant pools or in moist ground, which reaches maturity a short period after the laying of eggs by the female. Mosquitoes of the genus *Ochlerotatus* fulfil these conditions. The larvæ are found in small waterholes, such as the footprints of animals, where they develop into adults which are on the wing seeking a host within the week (5 to 6 days as larvæ and 2 days as nymphæ). The eggs are known to remain alive for a considerable time in moist earth. The insect would, therefore, act both as reservoir and as vector. The horse-sickness organism would thus not be an obligatory parasite, and would be accidentally transmitted by an insect feeding on a susceptible equine. Despite numerous attempts, no experimental proof of this theory has been adduced. It is very difficult to breed these mosquitoes in captivity and even more difficult to make them bite horses. The question of the natural transmission of horse sickness is, therefore, still unsolved and most urgently requires solution.

Pathological Anatomy.

Three forms of horse sickness may be distinguished: (1) the pulmonary, (2) the anasarcous or cardiac, and (3) the mixed form.

(1) *The pulmonary form.* The striking lesion of this form is œdema of the lungs, usually accompanied by hydrothorax. In typical cases the interlobular and the subpleural tissue is infiltrated with yellow liquid; the septa and the subpleural tissue are distended, transparent and gelatinous. The more acute the case, the more gelatinous is the consistency of the affected tissues. The lungs are nearly always inflated and in a state of acute emphysema; they are frequently distended to their full capacity, and remain fully distended on section. The œdematous

infiltration may also be found in the mediastinum and the base of the pericardium. The bronchial and mediastinal glands are swollen, soft and watery.

(2) *The anasarcous or cardiac form.* The striking lesions are found in the presence of an œdema in the subcutaneous, subfascial and intermuscular tissue of all parts of the body and in severe cases of all the lymphatic glands. In mild cases, mainly the head and neck are affected. These œdemas may be of such dimensions that they disfigure the regions involved. The muscular tissue of the affected parts is also infiltrated with the fluid. Another striking and almost constant lesion is the hydropericardium ; the amount of fluid may amount to two or more litres. The myocardium shows hydropic degeneration. Blood extravasations are practically never absent from the endocardium of both ventricles and reach large dimensions and are often found in the epicardium and in the heart-muscle.

(3) *The mixed form.* In this form, both pulmonary and cardiac lesions, as well as an œdematous infiltration may be found together.

Symptomatology.

Clinically four different forms may be distinguished: (1) the horse sickness fever, (2) the pulmonary form (known in South Africa as Dunkop), (3) the anasarcous or cardiac form (Dikkop), and (4) the mixed form.

The disease has a distinct incubation period. Observations in the field indicate that it is generally short. The usual experience is that nine days after the first frost the enzootic or epizootic comes to a standstill. As a general rule the incubation period in experimental horse sickness lasts from 5 to 7 days. The fever runs the same course in all forms of the disease. It is a typical remittent fever. Depending on the virulence of the virus, the stadium incrementi rises slowly or quickly, every successive day one or two degrees until the acme is reached. At this stage the disease may end fatally with pulmonary lesions, or the fever may enter into the stadium decrementi to end in recovery or in death from the cardiac form of the disease. A moderate temperature may be present, lasting from 3 to 5 days, generally from 5 to 7 days ; thus the disease, inclusive of the incubation period, lasts on an average from 13 to 16 days. In the pulmonary form, the fever may last from 3 to 5 days, rarely longer, and the whole period of the disease is from 9 to 14 days, the average being about 12 days. Certain animals show symptoms of Dikkop with hardly any fever reactions.

(1) *Horse-sickness fever.* Horse-sickness fever is seldom diagnosed in natural cases. Some horses may show slight symptoms of illness, such as loss of appetite, increased redness of the conjunctiva and a moderate polypnœa and accelerated pulse-rate. The temperature may rise to 105° F., but is usually lower, the acme may last from 1 to 3 days, rarely longer. Horse-sickness fever is the only form of horse sickness that can be artificially produced in the ass and the Angora goat. It is the usual form in horses and mules undergoing immunization.

(2) *The pulmonary form (Dunkop).* The pulmonary form of horse sickness (Dunkop) in its extreme condition shows all the symptoms of an acute pulmonary œdema in its final stages, followed as a rule by death. It is characterized by severe dyspnœa, paroxysms of coughing and discharge of frothy liquid in large quantities. The onset of the dyspnœa is generally very sudden and death usually occurs within a few hours. In another form the symptoms develop gradually, but in place of a single paroxysm there are two or even more. The animal may during such a paroxysm discharge large quantities of froth, the dyspnœa then subsides for a while and returns in the form of a second or third paroxysm. Recovery is rare; it follows a paroxysm and may be quite rapid. The pulmonary form can be diagnosed in the dog, and is characterized by distressing dyspnœa.

(3) *The anasarcous or cardiac form (Dikkop).* The anasarcous or cardiac form (Dikkop) represents the subacute form of horse sickness. It begins like the others; the fever reaches its acme usually a little later than in the pulmonary form, and may remain at its maximum for 2 to 4 days, and then descend slowly. The acme is reached about the twelfth or thirteenth day, and is succeeded by either a lysis, a slightly protracted crisis, or a critical descent. The latter is frequently the precursor of a collapse and the former two are often followed by a slightly subnormal temperature lasting one or two days. The œdematous infiltration of the tissues usually begins during the stadium decrementi and is fully developed at the end of the lysis or crisis. With the increase of the swelling the symptoms of cardiac dyspnœa develop, and the animal may now show distressing symptoms resembling those of the pulmonary form. The onset of the disease is frequently accompanied by cyanosis, noticeable in a dark-red conjunctiva, and a blue discoloration of the mucous membranes of the mouth (bluetongue).

(4) *The mixed form.* The mixed form is probably more often diagnosed at autopsy than during life, because lung lesions may also be present in the anasarcous form, but may not have developed sufficiently to influence the clinical picture. There are two main varieties: in the one the pulmonary symptoms appear first, develop slowly, persist or even recede, and are followed by the œdematous infiltrations; the animal may succumb to a belated paroxysm of dyspnœa or to the anasarcous form. In the other, the disease begins with the anasarcous form, with œdematous infiltrations, and is suddenly cut short by an acute paroxysm of the pulmonary form, to which the animal succumbs. The mixed form is often the result of a double infection, and is occasionally seen in the process of immunization when two or three different viruses are used.

Immunity.

A certain percentage of asses possess a complete natural immunity. Mules recover more readily than horses and are less difficult to immunize than their dams. There is no proof that naturally immune horses or mules exist. The fact that in certain experiments some animals survive all tests without reacting might be explained as a natural immunity, but, since the previous history of such horses is usually unknown, their immunity may be the result of a previous infection and recovery, and thus be acquired. The immunity observed in foals that were *in utero* during a horse-sickness reaction must also be considered as acquired. Horses and mules that have recovered from horse sickness are in South Africa known as 'salted animals'. In this article immunity as a result of a recovery from the disease is considered. The term resistance has previously been used to describe a quality of certain animals which do not react to a given quantity of virus, but which nevertheless remain susceptible to the same virus. Immunity in horses and mules is of a very variable nature. Many horses which have recovered from a natural attack or from an artificial infection of the disease will survive exposure during all horse-sickness seasons even in the worst localities. A considerable percentage of immune horses are known to contract the disease a second time when exposed to natural infection, and they are said to suffer relapses. Recovered horses are not virus carriers or for only a short period. Every attack is a new infection. Such reinfections may occur in one and the same season. To what extent outbreaks of the disease in immunized and susceptible horses occur under natural conditions is shown in Table I, which gives the collected results over a number of years and for various localities.

In this table the number of breakdowns refers only to those forms of horse sickness easily diagnosed at a distance, viz. the pulmonary and the anasarcous forms. Of these, the former was but rarely noticed and of the 64 breakdowns in 1917–18, only 2 cases succumbed to the pulmonary form and no more among the exposures of 1922–3. The horse-sickness fever as a breakdown to natural infection is therefore not registered. Leaving out the number of exposed horses, in which breakdowns were not recorded but only the deaths (Zululand, 1916–17), 793 exposed horses come into consideration. Of this number 175 horses, or 22 per cent., had breakdowns, and there were 81 deaths; the deaths were thus 10·2 per cent. of the number exposed, and 46·2 per cent. of the breakdowns. In view of the considerable number of horses exposed over a number of years, these figures can be regarded as giving a fairly correct idea of the protection recovery from horse sickness gives against subsequent infection. Compared with these figures, the result of the 1922–3 exposure experiment is very bad and represents the maximum of breakdowns so far recorded. There were 30·9 per cent. breakdowns with 17·3 per cent. deaths of the total number exposed or 56 per cent. of the breakdowns. It is also of interest to compare the immunity of fortified or hyperimmunized horses with that of unfortified horses, exposed to infection in different years. Of 175 such horses 17·7 per cent. had breakdowns, and 10·3 per cent. of the total, or 51·4 per cent. of the breakdowns, died. In experimental infections of horses kept under temperature control, the horse-sickness fever form is also recorded. In one such experiment immune horses were

TABLE I.

Immune and Hyperimmune Horses and Mules exposed to Natural Infection.

Year.	Place.	Immune and Hyperimmune Animals.					Hyperimmune Animals.				
		Number of Animals.*	Breakdown.		Deaths.		Number of Animals.	Breakdown.		Deaths.	
			Number.	Per cent.	Number.	Per cent.		Number.	Per cent.	Number.	Per cent.
1897–98	Elandshoek	18 H	6	33·3	2	11·1					
1897–98	Elandshoek	10 CH	10	100	10	100					
1905–06		3235 M	66	2	21	0·64					
1914–15	Onderste Poort	101 H	16	16	10	10	25			3	12
1916–17	Onderste Poort	224 H	14	6	8	3·5	79	5	6	2	2·5
1916–17	Zululand	50 H			4	8	23			2	9
1917–18	Onderste Poort	208 H	64	30	19	9	9	1	11	0	0
1922–23	Kaalplaats (near Onderste Poort)	242 H	75	30·9	42	17·3	87	25	28·7	16	18·4
1922–23	The mortality among immunized private horses exposed to natural infection was 17·6 per cent.										

H—Horses. CH—Control Horses. M—Mules.

*—Numbers in this column include hyperimmune animals which are also listed separately under the heading Hyperimmune Animals.

tested with different viruses. Of 73 breakdowns 18 were of the anasarcous form (24·6 per cent.) and of these 8 died (10·9 per cent. of the breakdowns). The rest of the breakdowns suffered from horse-sickness fever (75·4 per cent.). On account of the striking lesions, the anasarcous form is considered a severe form of the disease. In experiments with 90 horses that had breakdowns with lesions of the anasarcous form, 58 had previously recovered from the same form (64·5 per cent.) and 32 from the horse-sickness fever form (35·5 per cent.). Of 34 horses succumbing to the breakdown (anasarcous form) 28 had previously recovered from the same form (82·4 per cent.) and the remaining 6 horses (17·6 per cent.) had suffered from horse-sickness fever. It seems, therefore, that recovery from the severe anasarcous form offers less protection than does recovery from the mild horse-sickness fever. Of interest also is the comparatively rare occurrence of a second breakdown. Of 194 horses, recovered from the anasarcous form, produced by a single injection of virus, 46 (23 per cent.) again showed an anasarcous reaction (breakdown) when tested with a different virus. Of these 46 horses, only 2 showed a third anasarcous reaction when tested a second time with another virus. Probably these figures do not represent the true state of affairs, because the power of breaking the immunity is greater for some viruses than for others.

The Immune Serum.

The observations collected concerning the protective qualities of the serum of a recovered horse can be summarized as follows:

1. The serum of horses, mules and asses that have recovered from horse sickness has no protective qualities when injected, even in large quantities, before, simultaneously with, or after, a subcutaneous or intrajugular injection of a virulent virus.

2. The serum of horses, mules and asses which have been hyperimmunized has protective properties. The hyperimmune serum of horses is more protective than that of mules, and the latter is more effective than the serum of the ass. Serum of mules and asses, being unreliable, was no longer used in later experiments.

3. The serum of horses recovered from a severe anasarcous form of the disease has no better protective qualities than that obtained from horses which had only suffered from horse-sickness fever.

4. A mixture of serum and virus may not be virulent, either on intrajugular or subcutaneous injection, (*a*) when the proportion of serum to virus is large (100 c.cm. to 1 c.cm. of virus); (*b*) when the mixture has been kept for some time.

5. The serum of a fortified horse, when injected simultaneously and subcutaneously in quantities of 300 c.cm. or more, may prevent a reaction, (*a*) of a weak virus, even when injected in large quantities of 5 c.cm. or more; (*b*) of a strong virus when injected in quantities of less than 1 c.cm.

6. The serum of a fortified horse, when injected intrajugularly, may prevent the reaction of a weak virus simultaneously and intrajugularly

injected (3 c.cm. or more). It does not prevent a reaction when a stronger virus is used, even in smaller quantities (1 c.cm. or less).

7. The injection of hyperimmune serum before the virus may prevent the reaction of a weak virus injected from 1 to 7 days after the serum, but will not prevent the reaction of a strong virus.

8. The injection of hyperimmune serum after the virus may prevent the reaction when the serum is injected from 1 to 3 days after a weak virus, but not when injected after a strong virus.

9. There is no immunity in horses unless they have undergone a horse-sickness reaction.

10. There is no constancy of results in any of the above experiments undertaken at various times with viruses and serum from different horses.

11. The simultaneous intrajugular injection of virus and hyperimmune serum is followed in mules by a reaction and recovery in the majority of cases, if the serum is given in quantities of not less than 300 c.cm. per animal.

12. There is no constancy of results in horses treated in the same way. There are viruses that will give a reaction with recovery, when injected intrajugularly with sufficient serum, in a great number of cases, but never in all cases.

13. The reaction in horses injected intrajugularly with a selected virus and sufficient hyperimmune serum (0·75 c.cm. per kgm. body-weight) can be modified by a subsequent injection of hyperimmune serum (0·75 or 1·0 c.cm. per kgm. body-weight).

14. It is possible to combine the second serum with another intrajugular virus injection without increasing the risk of mortality, provided the second virus is an adequate one to the first virus. Under such conditions two reactions are frequently noted, overlapping or succeeding each other, and an immunity is produced that holds for both viruses.

15. It has not yet been possible to add a third virus to a third intrajugular serum injection following the second after an equal interval of time. The reaction is either too severe, the mortality too great, or no immunity is established to the third virus. In the latter cases, the quantity of serum injected prevents the third reaction.

16. Mules generally behave differently to horses. This is particularly the case with the simultaneous intrajugular injection of serum and virus, to which all susceptible animals react and only a small percentage die. Non-reacting mules are generally already immune.

Immunization.

Immunization may be obtained by vaccination, e.g. by using an attenuated virus as vaccine. The first method introduced into practice in South Africa was by Edington (1900) in the Cape Colony, who used a vaccine for both horses and mules. The method was comparatively safe for mules and produced immunity in the majority of cases, but was less successful in horses. The method was later withdrawn without Edington ever publishing anything about the process employed for the attenuation of the virus. A method of vaccination has also been introduced by Bevan (1918) in Rhodesia. In his annual reports he published from time to time the results of immunizations, the number of casualties succeeding vaccination and the breakdowns from natural exposure. He also has so far not published his method of attenuation. The method devised by Koch (1904) is only of historical interest and failed in practice. The method introduced by the writer in 1905 (Theiler, 1907[1, 2 & 4], 1909[1, 2 & 3]) was in the first instance devised only for the immunization of mules. It consists in the intrajugular injection of a virus (virus O), and the simultaneous injection of not less than 300 c.cm. of serum (serum O) for the average-sized mule. The reactions as a rule are mild and the mortality low. Calculated on a large number of mules for the last few years, it has been less than 1 per cent. Since the introduction of the method in 1905–6, 51,516 mules have been inoculated. The immunity obtained is better than that in horses, although it is also subject to various fluctuations. A fair idea of the protection mules receive by the immunization was obtained in 1905–6, when accurate statistics were collected. The breakdowns then recorded were 2 per cent. and the mortality 0·4 per cent.

The immunization of horses consists in the simultaneous intrajugular injection of a suitable virus with a sufficient quantity of hyperimmune serum (not less than 0·5 c.cm. per kgm. body-weight), and after an interval of a few days another intrajugular injection of virus and serum (not less than 0·75 c.cm. per kgm. body-weight). Two methods were worked out. In the first, which was later withdrawn, the virus of the first injections was the attenuated strain of the T virus, together with serum O. For the second injection, after an interval of six days, virus O and serum O were used. Owing to the unreliability of the virus T, which varied in virulence almost from animal to animal from which it was collected, and owing to the instability of its tenacity it was replaced with a virus of a different strain (virus N). The method was modified at the same time by shortening the interval to three days. This method is called the succedaneous or progressive method of immunization. The T and O method was introduced in 1910 and was in use till 1922–3. The N + O method was first used in the field in 1924–5, when a total number of 2,246 horses were inoculated. The casualties from the inoculation amounted to 3 per cent., being the best result ever obtained in horses.

Technique of Serum Preparation.

In order to obtain a standard hyperimmune serum a great number of horses are used. They are first immunized and then fortified by the transfusion of about 10 litres of virulent blood from a sick animal. The first transfusion is not made until a month after the recovery from the disease. Immune horses do not show any horse-sickness reaction after the transfusion of this large quantity of virus, which might amount to 10,000,000 minimum lethal doses of 0·001 c.cm. The transfusion is made by inserting cannulas into the jugular veins of both horses and connecting them by rubber tubing. The vein of the virus donor is compressed and the blood runs in a steady stream into the vein of the immune horse, at the rate of about 500 c.cm. per minute. The length of time the horse is transfused at one period varies, but frequently not more than two periods of 10 minutes are required to complete the process. Two weeks after the first transfusion the horses are tapped and the bleeding is repeated four times at intervals of five days. The average quantity collected from a horse is about 10 litres at one period. Subsequently the same process of hyperimmunization and bleeding is repeated twice, after the horses have had a rest of from 3 to 4 weeks. The serum is stored in a cold room, and later the serum of all the bleedings is mixed with the addition of some carbolic acid. This mixture contains serum from horses which were fortified from 1 to 3 times. The number fortified each time is about 30, so that the final mixture contains the serum from about 90 horses.

The transfusion is not supported equally well by all the horses. Some succumb with symptoms of shock or disturbances indicating some toxic effect of the blood of the donor on that of the receiver. Death in such cases takes place during or soon after the transfusion, rarely later, and never after 24 hours. Careful transfusion and stopping of the flow as soon as symptoms of distress are observed usually saves the horse. In some horses the transfusion is followed by the appearance of isolysins. Such serum, when injected, causes a dissolution of the blood of the injected horse, which frequently succumbs with symptoms of hæmoglobinuria and acute icterus. The effect of such serum is usually noticed within a day or two after the injection, but sometimes it is delayed and appears simultaneously with the fever. In these circumstances it is apparently the combined effect of the isolytic serum and the toxin which produces the hæmolysis. This danger can, however, be averted by testing the serum of each horse for its hæmolytic effect *in vitro*. For this purpose, a certain quantity of washed red corpuscles of a number of susceptible horses are suspended in a saline solution, and a specified quantity of the serum to be tested is added. The serum of horses showing a marked dissolution is then discarded. The number of horses that become hæmolytic is comparatively small, about 4 to 5 per cent.

Technique of Immunization.

Mules receive the minimal quantity of 300 c.cm. serum into the jugular vein on one side and 3 c.cm. of virus on the other side. The animal cannot be worked for at least three weeks, during which time it passes through the disease. The fever reaction is usually over by the fifteenth or sixteenth day. Horses receive a quantity of serum according to their size, 0·5 or 0·75 c.cm. per kgm. body-weight for the first, and 0·75 to 1·0 c.cm. per kgm. for the second injection. Both injections are intrajugular, as well as that of the corresponding virus, which is given in quantities of 5 c.cm. N virus is given the first day with 250 to 350 c.cm.

serum, followed by O virus on the third day with 300 to 450 c.cm. serum; thus the total quantity of serum used for a horse is from 550 to 800 c.cm., or more for very heavy horses. The injections are well supported, but there are accidental deaths due to the intrajugular injection, varying with the care of the operator. Injected horses should be protected against inclement weather, they should be stabled if possible and not worked for at least four weeks. Pregnant mares should not be injected as they frequently abort and succumb to the disease.

Curative and Preventive Treatment.

So far, all attempts to find a specific drug have failed. The injection of serum into a horse suffering from the pulmonary or from the anasarcous form is of no value.

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CHAPTER XXXII. DENGUE AND PAPPATACI FEVERS.

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Dengue Fever.

DEFINITION, EPIDEMIOLOGY AND ANIMAL SUSCEPTIBILITY.

Definition.

DENGUE FEVER is a mosquito-borne disease, rarely fatal, but frequently producing much general discomfort ; it is sometimes mild. It is characterized by sudden onset, a variable and usually diphasic febrile course of 3 to 8 days' duration, headache, aching eyes, severe body and limb pains, frequently a relatively slow pulse, leucopenia, and a late rash of morbilliform or scarlatiniform appearance ; it is often followed by a prolonged convalescence.

Epidemiology.

From time to time dengue fever assumes epidemic proportions, the extent of the epidemic depending upon the distribution of the mosquito *Aëdes argenteus* (*Stegomyia fasciata*), the principal transmitting agent, and the seasonal abundance of this insect, though its distribution is not co-extensive therewith. Where *A. argenteus* extends into temperate regions, dengue fever may spread from areas nearer the equator during the warmer weather when mosquitoes are abundant, and die out completely for periods of years with the onset of winter. In tropical parts, once introduced, infection smoulders, to flare up later in epidemic form. There is no evidence that the disease is contagious or transmissible by fomites.

Animal Susceptibility.

There is no evidence that guinea-pigs, cats, dogs or other domestic animals have contracted dengue naturally during an epidemic in the course of which they must have been frequently bitten by infective mosquitoes. At the hands of most investigators infective blood has failed to produce recognizable reactions in guinea-pigs, white mice, rabbits, hogs, dogs and monkeys (*Macacus rhesus* and *M. sinicus*).

Guinea-pigs. Many observers have inoculated these animals with infective blood with negative results. In one case the blood of such an inoculated guinea-pig, after allowing for a reasonable incubation period, was injected into a human being, with a negative result. The results of Koizumi *et al.* (1917), who found dengue fever blood lethal to guinea-pigs in from 7 to 36 days, have not been confirmed. Harris and Duval (1924) carried out many experiments, obtaining as they thought 42 positive reactions in guinea-pigs with material derived from 11 human beings. As they obtained positive results with blood from 3 patients on the seventh day of the disease, and from 2 on the tenth day, periods when it would appear the virus of dengue is no longer present, doubt must be thrown on the interpretations they have made, especially since Siler *et al.* (1926) failed completely to confirm their work. Harris and Duval's guinea-pigs, after an incubation period of 3 to 5 days, had temperature reactions and leucopenia. They believed that they had conveyed the disease in guinea-pigs for as many as 45 generations. In cultures they found globoid bodies which also gave rise to a similar reaction. A paratyphoid infection which was present amongst their animals probably explains the results.

Recently, Blanc *et al.* (1928), at Athens, have carried out interesting experiments on animals by removing infective blood from a patient and injecting part into another volunteer to prove its infectivity, and part into a test animal. They had observed in man that infection might occasionally occur without the subject showing evidence of the disease, and they thought that perhaps the same might occur in animals. As man is the only animal definitely known to react to the virus, such cryptic infections could only be detected by the introduction of infective material from the test animal back into a human being. When, therefore, in their experiments the human control reacted to the virus they removed blood from the test animal inoculated at the same time, and injected the serum into another volunteer and also into an animal of passage. In this way in guinea-pigs, in two experiments out of six, they were able to show the presence of the virus in materials obtained on the fifth day after the inoculations of the guinea-pigs. In the one case, defibrinated blood injected subcutaneously gave rise to a typical attack of dengue after an inoculation period of nine days, confirmed by transference to another subject who reacted after six days, and this was followed by two further human passages. In the other case, 2·5 c.cm. of serum injected intravenously gave rise to typical dengue after six days' incubation, confirmed by further passages. The guinea-pigs thus harbouring the virus showed no febrile response or other evidence of illness. Subinoculations from them into further guinea-pigs failed to show infection of these by tests made into human beings. By similar means they were unable to detect the presence of the virus in pigeons, cocks, white rats, rabbits, a dog and a monkey (*Macacus sinicus*).

Rabbits. Couvy (1922), at Beyrouth, experimented with what he considered to be dengue but which may have been pappataci fever. He inoculated rabbits intraperitoneally with infective blood and obtained a febrile reaction after 4 to 7 days, followed by a second later rise in temperature. By grinding up the bodies of *Phlebotomi* and injecting them intraperitoneally and subcutaneously into two rabbits, he also obtained a reaction. He records the finding of a few spirochætes in the blood of his patients and of the rabbits. These results have not been confirmed. The spirochæte-like bodies, if not artefacts, may have been due to complicating infections.

We may sum up by saying that it seems probable that the virus of dengue fever affects man only and is not capable of causing a reaction in other animals. It may temporarily reside in guinea-pigs without causing illness in them, but has not been conveyed from one such animal to another in series.

EVIDENCE THAT THE DISEASE IS DUE TO AN ULTRAMICROSCOPIC ORGANISM.

By the injection of blood from an infected human being into a susceptible person the disease can be produced. As careful examinations of the cells of such blood and of its plasma or serum have failed to reveal the presence of any body which can be generally accepted as a parasite, and as examinations of *A. argenteus* known to be infective to man have also failed to show the presence of microscopically visible parasites, the ultramicroscopic nature of the causative organism may be inferred. As further support to this view the virus has been shown to be present in Lilliput, Pasteur-Chamberland, Chamberland L2 and L3 and Berkefeld N filtrates of infective blood, adequate controls being employed to show the reliability of the filters.

EVIDENCE OF FILTERABILITY.

Ashburn and Craig (1907) passed diluted infective blood through a Lilliput diatomaceous filter-candle and injected the filtrate into a volunteer, with successful results. Cleland *et al.* (1918, 1919), with filtrates obtained from Pasteur-Chamberland F candles, obtained four successful results and eight negative results. One of the positive results was not controlled as to the imperviousness of the filter to bacterial organisms. In the other three cases, the material filtered consisted of citrated blood, serum and whole blood respectively from patients 22, 67 and 47 hours after the onset. In the first of these patients the attack was mild and without a rash, but in the other two quite typical with characteristic rashes. Of the eight negative cases, four of the results were explainable as due to absence of the virus from the original material owing to the late stage of the disease, another to the virus having been kept outside the body for eight days in the cold and having deteriorated in consequence, whilst in the remaining three, the virus was considered as having been present before filtration and as having been retained by the filter owing to slow filtration and plugging of the filter pores.

Blanc *et al.* (1928) have recently shown that the virus in infective serum diluted with physiological saline solution can pass through the pores of Chamberland L2 and L3 bougies, and Kligler and Ashner (1928) have been successful with serum passed through a carefully controlled Berkefeld N filter.

Manoussakis (1928) has transmitted the disease to volunteers with filtrates from Chamberland L7 and L11 bougies. The dengue thus conferred was typical of the natural disease and successive passages led to no modification of the reaction to the virus.

NATURE AND PROPERTIES OF THE VIRUS, ACTION OF PHYSICAL AND CHEMICAL AGENTS, TRANSMISSION EXPERIMENTS AND ARTIFICIAL CULTURE.

Nature and Properties of the Virus.

Most observers, after prolonged search of infective blood and of the organs of animals after inoculation with infective blood, have failed to find spirochætal or other organisms by dark-ground illumination, by staining methods, or by cultivation. Beyond being a filterable organism, the real nature of the virus still remains unknown. By some a relationship has been supposed to exist between dengue fever and yellow fever. This is chiefly based on both diseases having, as their principal vector, the same species of mosquito, *Aëdes argenteus*, and on the behaviour of the virus in man and in the mosquito, as shown experimentally—its incubation period in man, its presence during the first few days of illness and then its disappearance, and the length of the incubation period in the mosquito (Siler *et al.*, 1926), which then remains infective probably for the rest of its life. Clinically, the resemblances between the two are less striking, a characteristic rash being absent in yellow fever and lesions in the liver and kidneys not occurring in dengue. It is conceivable, however, that a mutation in the virus of one or the other or evolution from a common stock in two relatively isolated races of mankind, might account for such differences as now appear. If the viruses of dengue fever and yellow fever are thus phylogenetically closely related, the detection of the causative organism of one of these diseases will probably be quickly followed by the recognition of a similar organism in the other. As it now seems probable that the *Leptospira icteroides* of Noguchi is not the cause of true yellow fever, the likelihood of dengue being due to a *Leptospira* is greatly diminished. The claims for such must, however, be discussed.

Claims for a Spirochætal Organism.

The work of Fletcher (1928) on leptospirosis in the Federated Malay States, a disease distinct from dengue and not infrequently showing evidence of jaundice, explains the finding by Vervoort (1922, 1926) of his *Leptospira pyrogenes* in a dengue-like fever in Sumatra, in which jaundice was frequently noted. Couvy (1922) reported finding spirochætes, few in numbers, at the beginning of the fever in an epidemic in Beyrouth in 1920 and 1921, which he thought was dengue, but which may have been pappataci fever. These bodies had two or three turns in the spiral. They were found also during the resulting fever in rabbits inoculated with the blood of patients or with the triturated bodies of *Phlebotomi*. Carbo-Noboa (1924) in Ecuador described *Leptospira asthenoalgiæ* in the blood, cerebrospinal fluid and organs of patients suffering from ‘ Spanish dengue ’. The *Leptospiræ* were 2 to 7μ long and he obtained positive Pfeiffer's and agglutination reactions. It seems probable that the bodies of Couvy and of Carbo-Noboa are either the *Leptospiræ* referred to by Fletcher, or else pseudo-spirochætes derived from red corpuscles or other cells.

Siler *et al.* (1926), by silver-impregnation staining of smears of the viscera of infective mosquitoes, found in several of these small, extremely delicate, spiral filaments in groups and uniform in size and shape. These bodies await fuller investigation. Recently Kligler and Ashner (1928) in Jerusalem seem to have disproved completely the *Leptospira* theory with infective materials obtained from Syria and Haifa. They failed to find *Leptospiræ* in the original material even after centrifugation for two hours at 3,000 revolutions, and in cultures on media suitable for growing these organisms. They showed that their methods were capable of detecting such organisms even if present in small numbers. The claims of a *Leptospira* as the cause of dengue can thus apparently be dismissed.

The Globoid Bodies of Duval and Harris.

The globoid bodies of Duval and Harris (1924) are probably not the causative agents of dengue. Siler and his co-workers (1926) could not confirm their results either in cultures or in guinea-pig inoculations, and found that normal blood gave rise to similar cultural appearances. Duval and Harris likened their supposed organisms to the globoid bodies found by Flexner in acute poliomyelitis. Their supposed positive results in guinea-pigs are open to much doubt, as successful results were obtained with blood taken as late as the tenth day in dengue, and a paratyphoid infection was present amongst their stock guinea-pigs.

Rickettsia.

Sellards and Siler (1928) have found numerous *Rickettsia* bodies in the hind gut of infected *Aëdes argenteus*, both free in the lumen and in smaller numbers in the epithelial cells. They were not present in the salivary glands or outside the alimentary canal. These bodies were not seen in control mosquitoes. Further experiments with a special stock of mosquitoes were contemplated by the authors. The situation of the *Rickettsiæ* in the hind gut would suggest that contaminated fæcal infection of the mosquito bites was the method of introduction, if these bodies are the causative agents of dengue. Such a method of infection does not seem likely.

The Introduction of the Virus into and its Location in the Human Body.

The virus is introduced under natural conditions into the human body by the bite of infective mosquitoes. Subcutaneous and intravenous inoculation of infected blood or blood derivatives give successful results. The application of infective serum to a scarified area and swabbing the nose with infective material have failed to convey the disease. Gargling the throat with such material followed by swallowing it has given a result which was probably negative. Manoussakis (1928) could not transmit the disease by the filtered washings from the nasopharynx or by filtrates of the urine, or by administration of infective material by the mouth. He obtained successful results in two subjects by introducing virus material into the nose at the rate of two drops every hour during two days. He found the virus present also in the cerebrospinal fluid though this showed microscopically no evidence of any reaction to it. The amount of blood introduced experimentally has usually been 1 or more c.cm., but Koizumi *et al.* (1917) have found that 0·00005 c.cm. was infective for man.

By direct inoculation of human beings the virus can be demonstrated in the blood as a whole, in the serum of clotted blood, in the fluid part of citrated blood, and in the filtrate of infective blood that has been passed through Lilliput diatomaceous, Pasteur-Chamberland F and other filters. Mosquito transmission also shows its presence in blood. Successful transmissions by using washed corpuscles may have been due to the adherence of the virus to the outside of the cells from which the washing had been unable to detach it. On the other hand, it is possible that the fluid part may have been infected by the escape of the virus from the cells. There is no evidence that the virus can escape from the human body in any other way than by the withdrawal of blood, the patients not being contagious or infectious and their fomites and surroundings being in no way dangerous to others except through the bites of mosquitoes infected from them. The virus can be transmitted directly by inoculations from man to man at least for four generations without alteration in the characteristics of the resulting disease. Experimental passage from man to mosquito and back to man through six generations (Siler *et al.*, 1926) did not lead to attenuation or increase of virulence in the virus. The clinical forms produced experimentally in this series embraced ' every conceivable clinical type ', indicating thus that the varying reactions manifested by patients to the virus would seem due to the idiosyncrasies of the patient rather than to differences in the strains of the virus.

Incubation Period and Duration of the Virus in the Human Body.

The incubation period by mosquito transmission (Siler *et al.*, 1926) is usually from 4 to 7 days, but may be extended to 10 days, or even delayed to the eleventh day in patients partially immune. Ashburn and Craig (1907), by intravenous inoculation of 20 minims of blood, had incubation periods varying from 2½ to 7 days. The short period of 2½ days has not been obtained by other investigators, even by intravenous inoculation. The incubation period, by the injection of blood either intravenously or subcutaneously, has varied from 3 to 8 days, with one doubtful result in 15 days. Siler and his co-workers (1926) have shown that the patient can infect suitable mosquitoes in the late prodromal stage of the disease for several hours before the appearance of definite symptoms, that he is most infective during the first day and that by the end of the third day he is usually no longer capable of conveying infection to the *Aëdes* (*Stegomyia*), though it seems possible that in severe cases infection may be prolonged to the fourth day. Ashburn and Craig (1907) got positive results with blood taken on the third and fourth days of the disease, and Cleland *et al.* (1918)

got successful transmission results with bloods taken between 18 and 90 hours after the onset, but negative results with blood taken 130 hours from the beginning of the disease. Manoussakis (1928) found the blood virulent from a few hours before symptoms first appear until defervescence occurs. There is no clear experimental evidence that the patient is still infective after the fourth day, a matter which it is important to remember in connection with some of the work performed in searching for the causative agent.

Action of Physical and Chemical Agents.

Cleland *et al.* (1918) showed that infective blood may retain its infectivity outside the body in a cool place for as long as 7 days. Blanc *et al.* (1928) have recently found that the virus may retain its activity in the dark in a cupboard at a room temperature of 15 to 18° C., giving typical positive results on intravenous injection after 5, 12, 23 and 54 days, in the last instance after dilution and filtration through a Chamberland L3 bougie.

Manoussakis (1928) has shown that heating infective material for half an hour to 50° C. destroys the pathogenicity as well as the antigenic (protective) properties of the virus which a temperature of 40° C. fails to do. A similar loss of both pathogenic and antigenic properties followed keeping the virus at room temperature (12 to 15° C.) for more than 8 days, or in the incubator or the ice-chest for more than 7 days. Exposure in an ampoule of white glass to sunlight for half an hour did not destroy the virulence but led to an attenuated attack of the disease.

Transmission Experiments.

By direct inoculation from case to case. As already indicated, direct inoculation from case to case has proved successful with blood and various derivatives of the blood including filtrates. Inoculations are successful both by the subcutaneous and intravenous routes. The resultant disease clinically resembles the naturally acquired disease. Like natural dengue, it may be sometimes reduced merely to a slight fever. Blanc *et al.* (1928) have shown that occasionally the infection may be 'inapparent' or cryptic, with no evidence of illness, the presence of the virus after the incubation period had passed being demonstrated by injection into a fresh subject who reacted typically. Such an experimental but inapparent infection confers immunity, as shown by reinjecting the subject with infective material controlled as regards its containing the virus, and then showing by a further human inoculation that now no reaction followed the injection of the blood of the 'inapparent' case taken at a time when the control was reacting. The virus is not weakened by passage from man to man.

By mosquitoes. The 'Yellow Fever' mosquito, *Aëdes* (*Stegomyia*) *argenteus* Poiret, 1787 (Syn. *A. ægypti* L. 1762, *Stegomyia fasciata* Fabr. 1805 and *S. calopus* Meigen, 1818) has been incriminated on epidemiological grounds and proved experimentally to be the vector of dengue in Australia, in the Southern United States and in the Philippine Islands; Siler and his co-workers (1926) obtained 47 transmissions in 111 biting experiments with this insect in the last-named locality. In Formosa, Morishita (1925) got positive results with *Aëdes albopicta* (*Stegomyia scutellaris* Walk.), as well as with *Armigeres* (*Desvoidea*) *obturbans* Walk. The mosquito-conveyed disease is identical with natural dengue. The male mosquito, not being a blood-sucker, cannot convey the infection.

Attempts to convey the disease by *Culex fatigans* Wied. (*C. quinquefasciatas* Say) have failed, except possibly in a case reported by Craig, and in this instance it seems probable that the disease was conveyed by the regurgitation of infective material into the wound made by the mosquito in biting the volunteer, as the mosquitoes in question had bitten the previous case only two days before. Negative results have been obtained (Carpenter and Sutton, 1905) with *Culex* (*Aëdes*) *stimulans* Walk., 1848, and *Culex tarsalis* Coquill, 1896.

A single suitable mosquito can apparently convey infection. Siler *et al.* (1926) got positive results from the bites of two *Aëdes* (*Stegomyiæ*). These workers found that the incubation period in the mosquito was usually from 11 to 14 days. They obtained negative results from the bites of *Aëdes* 7, 8, 9, 10 and in one case 11 days after they had bitten an infective patient, but a positive result in another case on the eleventh day. The negative results were controlled by obtaining positive results in other individuals with the same mosquitoes 17 to 20 days after they had had their infecting meal. They have proved that the mosquito remains infective from 11 to 75 days after the infective feeding, that is probably throughout the remainder of her natural life.

Schule (1928), in the Philippine Islands, has obtained successful transmissions with mosquitoes 8, 10 or more days after their infective feed, but not 2, 3, 4 or 6 days afterwards. He suggests that a higher temperature may have led to the virus maturing more quickly in his mosquitoes than in those used by Siler *et al.* (1926). He was unsuccessful in transmitting the infection by interrupted feeding, that is by allowing *Aëdes argenteus* to take part of their meal from a patient in the first day of dengue and completing the meal on a susceptible volunteer.

Chandler and Rice (1923), on the other hand, obtained positive results from *Aëdes argenteus*, 1, 2, 3 and 4 days after feeding on dengue patients in the second, third, fourth and fifth days respectively of their diseases, with incubation periods varying from 4 to 6½ days. These results, together with Craig's apparent transmission by *C. fatigans* on the second day after an infective feeding, suggest that there may be two methods of mosquito transmission, one requiring the development of the virus in the specific mosquito, and so an incubation period of 11 or more days followed by permanent infection, the other of the contaminant type with regurgitation of the still infective original material into the wound, and perhaps not necessarily requiring so specific an intermediate host. If the latter means of transmission is common, one would expect the occasional extension of dengue beyond the *Aëdes* zone into areas where *Culex fatigans* is alone present.

The female mosquitoes bred out from the eggs of infective ones gave rise in three experiments (Siler *et al.*, 1926) to negative results controlled by subsequently conveying the disease in other ways to the volunteers. The virus is, therefore, not hereditarily transmitted through the eggs of the mosquito.

Artificial Culture of the Virus.

Most observers with a great variety of culture media have failed to obtain any evidence of multiplication of the virus therein. Such results as those of Duval and Harris (1924) with their globoid bodies have already been considered adversely. The virus probably cannot be cultivated *in vitro* by any method at present known to us.

Acquired Immunity.

An attack of dengue is followed by a period of absolute immunity, which may be short (less than 53 days—Siler *et al.*, 1926) or may last from two to several years. It is probable that some very slight infections not recognizable as dengue may explain cases of apparent natural or racial immunity. The injection of infective blood into individuals who had had dengue 24 days, between 32 and 94 days (13 cases), and 229 days previously, has failed to convey the disease. Second typical naturally acquired attacks have been recorded after intervals of 201 days and of 2 years. Several cases have been noted after 2½ years. Epidemics of the disease in one locality are usually separated from each other by periods of from 3 to 10 years. In epidemics separated by seven or more years many have suffered from the disease again on the second occasion. Some white persons have had as many as four attacks. Second attacks have been produced by experimental inoculations on five occasions after intervals of between 53 and 121 days. In general, subsequent attacks are less severe and shorter than previous ones, and in native races, such as Filipinos, repeated reinfections may result in reactions in adults that are barely recognizable.

Manoussakis (1928) has found that the virus, when it has lost its pathogenicity as a result of heating or from age, fails to protect non-immunes into whom it is injected against the subsequent introduction of infective material. In this respect its antigenic power appears to have been lost, so that attempts at specific prophylaxis by means of a dead vaccine seem useless. He found also that the serum of tested immune convalescents, taken immediately or up to a month after the attack, was incapable of conferring any protection in doses of 5 to 15 c.cm., whether injected before or at the same time as the active virus. These experiments were repeated five times with the same negative results so that specific serum therapy, prophylactive or curative, appear to be equally futile.

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Pappataci Fever.

Synonyms : Phlebotomus fever, Three-day fever, Sand-fly fever.

DEFINITION, EPIDEMIOLOGY AND ANIMAL SUSCEPTIBILITY.

Definition.

Pappataci fever is a disease of short duration with or without tardy convalescence and practically never fatal; it is chiefly confined to tropical or subtropical countries, and is transmitted by sand-flies of the genus *Phlebotomus*. It is characterized by sudden onset, rapid rise of temperature with fall by lysis usually about the third day, headache, pains in the eyeballs and muscles, injected conjunctivæ, bradycardia and leucopenia with a large mononuclear increase. There are occasionally secondary rises of temperature. There is no distinctive rash.

Endemiology and Epidemiology.

The disease is essentially endemic, depending on the habits and distribution of the transmitting *Phlebotomi*. Specially favourable conditions may allow of a spread which is almost epidemic. There is a direct relationship between rainfall and the fever, as the breeding places of the *Phlebotomi* become dried up during prolonged heat. The insects breed in old rubble walls, in cracks and fissures, in caves and hollows, and suchlike places where there is slight moisture and organic matter combined with relative darkness. They do not fly far from these refuges, persons living in buildings within 50 to 80 yards of the breeding grounds being most apt to develop the disease, those farther away being rarely attacked (Young *et al.*, 1926). Heights above 7,000 ft. are inimical to the insects.

Animal Susceptibility.

No animal seems under natural or experimental conditions to suffer from the disease. Birt (1910) found monkeys, rabbits and guinea-pigs to be immune. Whittingham (1921, 1922) obtained a rise of 2° F. in temperature in guinea-pigs inoculated with 3 to 5 c.cm. of blood from sand-fly fever cases, but an epidemic of diarrhœa may explain this reaction. Couvy (1922) at Beyrouth experimented with cases he believed to be suffering from dengue. Pappataci fever seems to have been present there at that time and some of his cases may have been due to this disease. He obtained a double febrile curve (resembling some temperature charts of pappataci fever) by injecting rabbits with blood from his patients, but as he found a few spirochætes in both patients and inoculated rabbits, it seems possible that these were responsible for the reaction and were not connected with either dengue or pappataci fever in his patients. He obtained similar results by inoculating the ground-up bodies of *Phlebotomi* found in the neighbourhood of patients into rabbits.

EVIDENCE THAT THE DISEASE IS DUE TO AN ULTRAMICROSCOPIC ORGANISM.

In infective material such as blood, careful examination by modern methods in the hands of nearly all observers has failed to reveal the presence of any recognizable organisms. Spirochætal bodies seen by some are probably not the causative agents sought. The presence of the organism in ultramicroscopic form in the circulating blood must thus be assumed.

EVIDENCE OF FILTERABILITY.

Virulent blood diluted five times and filtered through a close-grained Reichel filter gave rise to a mild attack of the disease, whilst similar filtration through a Pukell filter failed (Doerr, 1908). Birt (1910) obtained successful results with infective blood diluted nine times after passage through a Chamberland F filter, the amount inoculated being equivalent to 0·7 c.cm. of original blood. Graham (1915) refers to the infectivity of a Berkefeld-Reichel filtrate. Kligler (1928) proved that the virus could pass through a Berkefeld N filter. As small a quantity as 0·1 c.cm. of serum can produce infection.

NATURE AND PROPERTIES OF THE VIRUS, ACTION OF PHYSICAL AND CHEMICAL AGENTS, TRANSMISSION EXPERIMENTS AND ARTIFICIAL CULTURE.

Nature and Properties of the Virus.

As pappataci fever seems closely related to the group comprising dengue, perhaps yellow fever, and some obscure allied diseases, the virus will probably be found to possess similar properties in general to those of these diseases. As yet no morphologically recognizable phase of the virus is with certainty known. The claims of spirochætal organisms will be considered shortly. The subcutaneous inoculation of 0·5 to 3 c.cm. of blood or serum taken from the peripheral circulation during the first 24 hours of the disease is capable of causing an infection in a susceptible person with an incubation period of 3½ to 7 days (Doerr, 1908). Under natural conditions the incubation period may apparently be as short as 2 days. Filtration shows that the virus may be present in the plasma and is not (at least permanently) endoglobular. The virus disappears from the circulation within 48 hours of the onset of the disease, so that blood taken on the second or later days of the disease is non-infective. Ophthalmo and skin reactions (Birt, 1910) with virulent blood gave negative results, and mixtures of serum from patients in the course of the disease, with that of convalescents, resulted in no evidence of precipitation.

The claims for a Leptospira. Whittingham (1921, 1922) detected the presence of a *Leptospira*, morphologically resembling *L. icterohæmorrhagiæ*, but differing in its lack of pathogenicity to guinea-pigs, in 6 out of 26 cases of what he believed to be sand-fly fever. He cultivated the organism but could not recover it from inoculated guinea-pigs. As jaundice developed in one of his 'sand-fly fever' patients, it seems almost certain that amongst his cases were patients suffering from the leptospirosis recently worked out by Fletcher (1928) in the Federated Malay States, and that these yielded the organisms. Whittingham himself thought that it was just possible his *Leptospira* was a modified form of that which causes epidemic jaundice.

The same explanation probably applies to the findings of Couvy (1922) in Beyrouth. He detected spirochætes, always few in numbers, in cases he thought were examples of dengue, though his account suggests they may have been due to sand-fly fever. These spirochætes were found also in rabbits inoculated with blood from the patients and with ground-up *Phlebotomi*, but again few in numbers. It is probable the organisms were not related either to dengue or to sand-fly fever.

Kligler and Ashner (1928) were unable to detect *Leptospiræ* in material known to be infective, either by direct examination, by various staining methods, by centrifugation, or by cultural methods suitable for these organisms. They failed also by similar means to detect them in *Phlebotomi* caught in infected houses. They showed that their methods could have detected *Leptospiræ* if present even in small numbers.

Action of Physical and Chemical Agents.

The virus in a filtrate has retained its infectivity *in vitro* for a week when kept in the dark at a temperature of 75 to 80° F. (Birt, 1910). It is destroyed at 55° C. in 10 minutes (Lépine, 1927). Injections of novarsenobenzol on account of syphilis failed to prevent the development of a typical attack of sand-fly fever (Young *et al.*, 1926)—a further point against the view that the causative agent is a *Leptospira.*

Transmission Experiments.

By direct inoculation from case to case. This is successful as already indicated with blood taken on the first day only of the disease.

By Phlebotomi. Females of sand-flies of the genus *Phlebotomus* are the vectors of the virus. *P. papatasii* Scopoli is certainly a transmitter. *P. perniciosus* Newst. and *P. minutus* Rondani have also been incriminated, to which Viskovsky and Petrov (1927) add *P. caucasicus* Marzinowsky and *P. li* Popow, the former of which Parrot considers as a synonym of *P. sergenti* Parrot, and the latter a variety of this species, *P. sergenti* var. *li.* As regards *P. minutus,* Young *et al.* (1926) could find no evidence that this species feeds on man. A single insect can infect (Birt, 1910). The sand-fly must bite the patient within 24 hours of the onset of the disease. After an incubation period of 7 days in the *Phlebotomus* this insect can convey infection, and the disease has been then conveyed from the seventh to the twelfth day. Thereafter the insect probably remains infective for the rest of her life, which in captivity is usually only 12 to 25 days, rarely as long as 46 days. Doerr's views as to transmission through the egg and larva have not been confirmed, but it appears (Whittingham, 1924) that larvæ may become infective by eating the dejecta of infected flies and that the imagines from these may transmit infection, though only after a feed of blood. Young *et al.* (1926) consider that 'to start the seasonal appearance of sand-fly fever in man, an infection of the insect in its breeding grounds must be postulated'. They found that female *P. papatasii* did not ordinarily die in the breeding grounds and that the bodies of those that do are not readily eaten by the larvæ, so as to infect them, when other food is plentiful. They suggest that possibly certain mites (*Raphignathus youngi* Hirst or *Trombidium hindustanicum* Hirst) found by them on sand-flies may act as vectors of the virus amongst these insects. When the temperature falls below 60° F., infected flies become non-infective.

ACQUIRED IMMUNITY.

New-comers to an infected locality are specially liable to contract the disease, an acquired immunity relatively protecting older residents. This probably explains the lessened liability to the disease of such races as the Chitrali and plainsmen amongst the Indian troops. The acquired immunity may, it is stated, last for life, though Lépine (1927) considers that it is of very short duration and that a lengthy immunity is certainly not the rule.

Second attacks sometimes constitute as much as 5 per cent. or 6 per cent. of cases and may occur within 3 to 8 weeks of the first one, and even third attacks in one season have been known (Lambert, 1918). Sometimes second attacks have not been altered in duration by the previous one, but Lépine (1927) considers that larval and abortive forms occur in indigenes and owe their characters to repeated reinfections since infancy. Whittingham (1922) reports relapses in 25 to 46 days in 12 per cent. of cases. Doerr *et al.* (1909) found that 0·5 c.cm. of virulent blood mixed with 1 c.cm. of serum from a patient who had had the disease two years previously failed to convey the disease, and a similar experiment with 1 c.cm. from a patient who had had pappataci fever seven days before, apparently delayed the onset. Four immune patients, injected by McCarrison (1915) with 2 c.cm. of virulent blood, failed to develop the disease.

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CHAPTER XXXIII. HEARTWATER.

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Synonyms: Veldt-sickness, Veld-Ziekte, Boschziekte, Galziekte, ' Brain-Water '.

Definition.

Heartwater is an acute septicæmic disease affecting sheep, goats and cattle in South Africa, produced by *Rickettsia ruminantium* (Cowdry). Recently Rickmann (1908) recorded its existence in German South-West Africa and Van Saceghem (1918) in Angola and the Belgian Congo. The disease is neither infectious nor contagious in the sense these terms are generally used. The mortality is high, between 70 and 80 per cent. According to Theiler (1905[1]) the disease is more frequent in the summer than in the winter months. It is transmitted from animal to animal by the bont tick (*Amblyomma hebræum*). The name ' Heartwater ' was originally given to the disease on account of a lesion frequently met with on autopsy, hydropericardium.

Ætiology and Transmission of the Disease.

The facts that heartwater is not contagious, and that when once animals were moved up to the high veldt cases ceased, had suggested that the bont tick, which only breeds on the bush veldt, might be the transmitting agent, and Lounsbury definitely showed that this was so, and that the nymphs and adults could carry the infection from one animal to another. Edington (1900) was the first to transmit the disease to susceptible animals by inoculation of blood containing the virus.

The evidence as to the filterability of the infective agent is conflicting. Theiler (1904) stated that the micro-organism responsible for the disease should come within the limit of visibility since its ability to pass through a Berkefeld filter had not been demonstrated. In 1905[2] he stated ' Le microbe n'est pas contenu dans le sérum, il parait adhérer aux hématies '. Later, in collaboration with Gray and Power (1914) he observed that the virus which is present in the blood-plasma passes through a Berkefeld filter-candle and must be of a very unstable nature, since its virulence is sometimes destroyed after 24 hours standing. Knuth and du Toit (1921) also stated that the virus, which is ultravisible, is present in the blood-plasma, and is capable of passing through a Berkefeld filter. Lounsbury and Robertson (1904) suggested that the causal agent circulating in the blood might be a protozoan parasite. Cowdry (1925[2], 1926[1]), at the suggestion of Theiler, made a study of the disease in experimentally infected animals to obtain evidence for or against the hypothesis that a Rickettsia might be the cause. He was able to demonstrate Gram-negative intracellular coccus-like bodies or Rickettsiæ in cases of heartwater in sheep, goats and cattle. These bodies are restricted in distribution to the endothelial cells of the smaller blood-vessels where they are found in large, densely packed masses. They are most easily detected in the renal glomeruli and in the small capillaries of the cerebral cortex. The presence of the micro-organism does not excite any detectible local reaction apart from the distention of the endothelial cells which contain them. Larvæ of the bont tick, when allowed to feed upon cases of heartwater, acquire Rickettsiæ which appear to be identical with those found in animals suffering from heartwater, whereas larvæ fed on normal animals remain free of Rickettsiæ. Moreover, when the infected larvæ moult, the resulting nymphæ contain Rickettsiæ in their alimentary tracts, and when they are allowed to feed upon susceptible animals produce in them typical heartwater. The tissues of animals thus infected are found upon histological examination to contain typical Rickettsiæ. The Rickettsia bodies have a diameter of 0·2 to 0·5μ when measured after fixation by Zenker's or Rigaud's fluid and stained by Giemsa's stain, or any basic aniline dye, and appear spherical in shape.

Although the Rickettsiæ are presumably present in the blood-stream during the febrile stages and for about six days after the first fall of temperature when the blood is known to be virulent, they have never been seen in blood smears. Similar negative results are obtained with fresh smears from various organs on autopsy. This failure corresponds with that experienced in examining the blood of cases of typhus fever and trench fever. The *Rickettsia ruminantium* is a very specific micro-organism and is confined to the bont tick. It could not be found in 24 other species of ticks examined (Cowdry). Cowdry (1925[1], 1926[2]) has described a group of micro-organisms transmitted hereditarily in various species of ticks which are apparently unassociated with disease. Their possible character and relation to Rickettsiæ are described by him.

Artificially, typical symptoms of the disease can be produced by the subcutaneous or intravenous inoculation of virulent blood taken from a sick or recently recovered animal (2 c.cm. or more). The inoculation of the fluid from the pericardial sac fails to produce the disease ; the injection of virulent blood from cattle into sheep and goats is not always fatal. After artificial infection, the incubation period is about 12 days when the disease is induced by ticks, and varies from 6 to 15 days as the result of the inoculation with virulent blood. The duration of the febrile stage is about 7 days.

Animals Susceptible and Animals Resistant to the Infection.

Sheep, goats and cattle are susceptible to the disease, but the susceptibility varies with the different breeds. The Angora goat is the chief victim. In cattle the disease is less severe than in goats and sheep.

Imported cattle are highly susceptible. The springbok has been said to be infected occasionally (Spreull, 1922). Lounsbury and Robertson (1904) found that Persian sheep take the disease readily when exposed to infection, but recover without serious illness, and as a result possess a high degree of immunity. They found that lambs bred from merino ewes by a Persian ram escaped the disease when the mothers died. The ticks from infected Persian sheep can transmit the disease to other breeds of sheep and to goats and cattle. The horse is not susceptible to heartwater.

Immunity and Prevention.

Although recovery renders an animal insusceptible to the disease for a period as long as 18 months in some cases, such acquired immunity does not appear to be absolute, and animals which have gone through an attack of heartwater may succumb to a second attack some months later when their blood again becomes infective (Spreull, 1904; Theiler, 1905^1). Hyperimmunization produces a serum capable of conferring a brief immunity.

Eradication of the disease by starving out the ticks is only possible provided all susceptible stock are removed from the farm. Such starving out would take over 20 months, and, therefore, is not practicable. Burning the grass is insufficient as ticks in crevices in the ground escape. The stocking of pastures with Persian sheep, which appear to be resistant, was suggested, but this is also impracticable as they still harbour the infective agent, and are a reservoir of infection for cattle and other susceptible stock.

Dipping of cattle alone is sufficient to eradicate the disease. Sheep cannot be as frequently dipped as cattle: the cattle must be used to collect the ticks, and dipping should be practised at short intervals. In areas where cattle are systematically dipped, heartwater disappears.

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CHAPTER XXXIV. TYPHUS FEVER.

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(*Synonyms:* Typhus exanthematicus, Typhus exanthématique, Flecktyphus, Fleckfieber, Tifo esantematico, Tabardillo.)

Section A.

BY J. A. ARKWRIGHT.

INTRODUCTION.

THOUGH the disease to which the name typhus is now applied has no doubt been present for many centuries in endemic form and has also been responsible for epidemics, until about a hundred years ago it was confused with other continued fevers. About the middle of the nineteenth century it was first clearly distinguished from relapsing fever by the presence of the spirillum of Obermayer in the blood in the latter disease and from typhoid fever by the pathological changes found *post mortem* in the intestinal walls in the 'enteric' group of diseases.

CLINICAL ACCOUNT.

Typhus fever is typically characterized by rapid onset with high fever, which lasts 12 to 14 days and usually ends with a sudden crisis; by a mottled rash on the trunk and limbs, which may be quite distinctive and may last from the fourth or fifth day throughout the fever or may be very slight and fugitive, and usually by a state of complete delirium. There is generally severe bronchial catarrh or broncho-pneumonia and often diarrhœa. The convalescence is slow but recovery is as a rule complete. In patients previously reduced by want of food and exposed to cold, gangrene of the extremities is of frequent occurrence. Very mild cases are not infrequent in places where typhus is endemic, especially among children, and persons who have suffered in previous epidemics may also have very slight attacks.

PATHOLOGY.

Post mortem the distinctive pathological changes in the organs and tissues are confined to microscopic nodules in the walls of the very small blood-vessels. These were first described in the skin by E. Fränkel (1914) and consist of aggregations of the endothelial and other mononuclear cells lining the smallest arterioles and aggregated around the vessels. These foci are also very constantly found in other organs and especially in the brain and kidneys (Aschoff, 1915; Jaffé, 1918; Wolbach, Todd and Palfrey, 1922). Spielmeyer (1919) has given a very complete account of the histology, and Ceelen (1919) has reviewed the literature of the subject.

Thrombosis occurs at the sites of the nodules. These are sometimes very numerous in the midbrain in the region of the *iter*. According to Wolbach, in addition to the definite nodules there is a characteristic diffuse infiltration of the skin. The nodules are found in the brain and other organs of infected monkeys and guinea-pigs as well as in man. After recovery the lesions may resolve without leaving permanent changes or a slight fibrosis may result. The larger blood-vessels may also be the seat of changes in the intima.

Typhus fever is usually associated with poverty, squalor and overcrowding, and it is still frequent in some countries in places where these conditions obtain. Formerly it was almost universally endemic in the larger towns of Europe, especially in the form of ' gaol fever ', and has often caused devastating epidemics in time of war. In Serbia, during the first six months of 1915, it is believed to have been responsible for 120,000 deaths, and huge epidemics have occurred in Russia and Poland during the late war and since.

Typhus has long been known in this country to be highly infectious, since brief contact with patients in their own homes has been recognized as an almost certain means of acquiring the disease. Often whole families were attacked and the disease also spread rapidly in prisons and among crowded, ill-attended patients in hospitals. Under such conditions doctors and nurses rarely escaped. During the epidemics of the nineteenth century in Edinburgh, Dublin and elsewhere, it was noticed that typhus did not spread in well-appointed hospitals and in these only porters and attendants occupied in the receiving-rooms were attacked.

Typhus fever appears to attack equally persons of both sexes and all ages. It is often very mild in children and the severity rises with age. According to Goodall (1920), in 1917 in Poland the mortality was 4·1 per cent. at 20 to 30 years ; 10 per cent. at 30 to 40 ; 16·7 per cent. at 40 to 50 and 52·6 per cent. at 50 to 60 years.

EPIDEMIC TYPHUS.

Epidemic typhus is a disease of cold and temperate climates and is especially prevalent during winter when the dwellings are more crowded and clothes are less often changed or washed. Whilst rare in Western Europe during the past fifty years, it has never ceased to be endemic in Eastern Europe, especially Galicia, Poland and Russia and in Northern Asia. Small outbreaks still occur occasionally in Ireland (King, 1927) and more rarely in Liverpool and Glasgow. In the United States, typhus has not occurred in epidemic form since 1892–3, though an endemic and epidemic centre exists in the highlands of Mexico.

SPORADIC TYPHUS AND BRILL'S DISEASE.

A certain number of sporadic cases of typhus, which are usually mild, have, however, been reported in the United States of America. Besides a few cases imported from Europe, there is an endemic form of the disease which was first described by Brill in New York in 1898, who, in 1910.

also reported 221 cases between 1896 and 1909. Maxcy (1926, 1928, 1929) has also recorded cases believed to be similar in the South Eastern States. These have been for the most part scattered singly, but altogether in the years 1915–27 295 cases were recorded from New York State, in 1912–27 210 from Alabama and 314 in Georgia. In 1924 twenty somewhat similar cases were reported by Sinclair and Maxcy (1925) in the Rio Grande Valley between Mexico and Texas. These sporadic cases are important from the point of view of ætiology and epidemiology, since in these respects they are not like typical typhus. They resemble the cases in Malaya described by Fletcher and Lesslar (1925, 1926).

Occurrences of an undetermined fever resembling typhus have also been reported from different parts of India, especially the Kumaon district, by Megaw (1921), from Nigeria by Davies and Johnson (1921), from Adelaide 37 cases by Hone (1922–3), from Queensland 38 cases by Wheatland (1926) and also from Kenya by Clearkin. In the south of France, especially near Marseilles, typhus-like cases of disease are known and have been investigated by Burnet and others (Burnet and Durand, 1929). Allessandrini (1929) reported similar cases from Rome.

The chief peculiarity of these atypical cases consists in the mildness of the attack, as shown by the very low mortality in most areas, by the absence of evidence of contagiousness, and by their sporadic occurrence. Their prevalence is also in the summer rather than the winter and they occur in warmer climates than is usual for epidemic typhus.

ÆTIOLOGY.

Little advance was made in the investigation of the ætiology of typhus till in 1909 C. Nicolle succeeded in transmitting the disease to monkeys and in 1911 to guinea-pigs. Before then, and, indeed, till the subsequent work during the War (1914–18), the search for the cause consisted in a series of unsuccessful attempts to isolate the infecting micro-organism.

Rabinowitsch, Hort, Penfold, Robertson, Plotz and Barykin all isolated bacteria from the blood in apparently pure cultures. In some instances, especially in the case of Plotz's bacillus, it was claimed that the patient's serum specifically agglutinated the bacterium isolated, but no disease at all resembling typhus could be produced in monkeys with any of the cultures, and no specific relation between these bacteria and typhus fever has been established. The conclusion was drawn from these diverse observations that the blood in typhus fever was often invaded by a variety of micro-organisms which were not the ætiological factor. The diphtheroid bacillus which was described by Plotz as the infecting agent and was also found by Olitsky and others, closely resembled bacteria commonly found on the normal human skin and also certain strains which have been isolated from the spleen and kidney of normal rabbits, which latter organs were usually components of the culture medium used for the isolation. Emulsions of this micro-organism were also very often too unstable for satisfactory agglutination tests.

As the aftermath of the immense epidemics in Serbia, Poland and Russia, &c., during the War, three important series of facts have become firmly established in relation to the ætiology and immunology. These represent three different aspects of the disease from which the problem has been approached. The exact interrelation of all the observations is not yet determined but some light has been thrown on the whole subject, though the ætiology is not yet by any means free from obscurity.

(*a*) The virus or infective agent is present in the blood and organs of man and animals during the acute stage of the disease, and when inoculated into a susceptible animal can reproduce the infection.

(*b*) The virus is present in the gut of *Pediculi* which have sucked the blood of typhus patients or monkeys, and from these lice, 8 or 10 days later, the disease can be passed to other hosts. In the gut of the infected lice an organism, *Rickettsia prowazeki*, is present in very large numbers.

(*c*) The blood-serum of typhus patients from the fourth or sixth day to the end of the fever and during convalescence will agglutinate an emulsion of *B. proteus* belonging to the special group of strains called by Weil and Felix, Proteus X. This reaction is so specific that it is established as a very valuable guide in the diagnosis of typhus fever.

An account of this group of proteus strains and their relation to the disease is given by Dr. A. Felix in Section B (p. 412) and by Dr. J. W. Wilson in Vol. IV, p. 308, where also the claims made by Wilson and others, that certain strains of coliform bacteria are agglutinated in a similar manner, are discussed.

The Virus in the Blood and Organs of Man.

C. Nicolle (1909), by the subcutaneous inoculation of blood, transmitted the typhus fever of Northern Africa from man to a chimpanzee. He further passed on the infection by means of the blood from the chimpanzee to four macaques in series. These monkeys also reacted with a well-marked rise of temperature. Anderson and Goldberger (1912) confirmed these experiments in North America, starting with blood from a case of typhus or tabardillo in Mexico City. Blood taken from man at the height of the fever in doses of 0·75 to 5·0 c.cm. has produced infection of monkeys when inoculated subcutaneously, intraperitoneally or intravenously. After an incubation period of usually 5 to 8 days, but occasionally prolonged to 24 days, the fever in monkeys begins either gradually or suddenly and lasts 4 to 8 days. As a rule the temperature rises 1·5 to 2·0° C. (3 or 4° F.) above the normal for the particular animal. Anderson and Goldberger infected in this way 23 monkeys in series. The illness in the monkey is as a rule slight and the mortality low. After recovery, the animals were found to resist a second inoculation without febrile reaction. A few monkeys proved to be naturally resistant. A few years later (1911) Nicolle, Conseil and Conor showed that the guinea-pig was also susceptible and that the virus kept its activity if passed through a number of these animals in series.

The virus is present in the plasma after the red blood corpuscles have been removed from citrated blood by centrifuging for a short time. Defibrinated blood obtained by shaking with beads is infective, but the serum was found by Goldberger not to be active, if it had been removed from the clot formed on standing. Nicolle and others (1912) showed that the virus was especially concentrated in the leucocyte cream obtained by centrifuging citrated blood and believed that it was intimately associated with the leucocytes. Kusama (1921) showed that an emulsion of platelets without leucocytes obtained by fractional centrifugation of citrated plasma contained concentrated infective material. This was confirmed for the guinea-pig by Ségal (1922) and Bacot and Ségal (1922). Weigl (1920) used washed leucocytes of man and guinea-pigs for infecting lice, and Bacot used the platelet emulsion for the same purpose. Reimann, Lu and Yang (1929) could not confirm the association of platelets and virus.

The symptomatology in animals and their experimental infection by direct transference of the blood and tissues will be dealt with in Section B by Dr. Felix.

Unity of Typhus Fever?

Epidemic typhus in all parts of the world has always been considered uniform in character and due to the same virus, though varying in severity. This view has been confirmed by means of cross-immunity experiments with monkeys, guinea-pigs and rats by Nicolle (1916), Netter and Blaizot (1918), Olitsky (1921) and Pinkerton (1929). The epidemic disease in different countries of the Old and New Worlds is also uniform as regards its great infectiousness in man, its transmission by lice and the Weil-Felix reaction given by the serum of patients.

Sporadic typhus. The sporadic cases resembling typhus which occur in various parts of the world are less typical in certain particulars. Associated with the rarity of any evidence of infection from one case to another, and the fact that the majority of cases occur in summer instead of winter, it is uniformly stated that the patients are free from body-lice and usually from head-lice. Most of the cases are, however, connected with epidemic typhus by the Weil-Felix reaction which is given with the blood serum of the patients.

Brill's disease in New York was identified by Anderson and Goldberger (1912) with Mexican typhus or tabardillo by means of cross-immunity experiments. The cases of sporadic typhus in the Eastern United States of America which are generally grouped with Brill's disease have been studied of late years by Maxcy (Sinclair and Maxcy, 1925; Maxcy, 1926, 1928), and Maxcy (1929[1, 2 & 3]) and Pinkerton (1929) have obtained strong evidence of the identity of strains of virus from the South Eastern States and Mexico by similar methods, using guinea-pigs and rats.

No cross-immunity experiments have been made with the cases reported from Malaya, Australia, India, Nigeria and Rome. Megaw (1921) compared the fever in Kumaon to Rocky Mountain fever and suggested that the undetermined fevers of the tropics would eventually be grouped as (1) typhus spread by lice, (2) Rocky Mountain fever by ticks, and (3) Tsutsugamushi by mites. Burnet, Durand and Olmer (1928) showed that the virus of cases from the neighbourhood of Marseilles was unlike that of African typhus.

Burnet and Durand (1929) reviewed the subject of sporadic typhus and agreed that the cases described under this name would probably eventually be found to fall into groups transmitted by different arthropods, and they believe that the 'fièvre exanthématique' of Marseilles is identical with 'fièvre boutonneuse' of North Africa and is transmitted by a tick.

Transmission by Lice.

In the eighteenth and nineteenth centuries it was recognized that in their own homes not only were patients themselves infectious but also their clothes, and that even healthy persons from an infected house might carry infection. The further knowledge that the virus was contained in the blood, and observations on the distribution of the disease, led Nicolle strongly to suspect the body-louse as the vector of the virus. This mode of transference had been suggested by the work of Mackie (1907) and of Sergent and Foley (1908) on the relation of *Pediculi* to relapsing fever. Nicolle also pointed out that if other blood-sucking insects, such as fleas, bed-bugs and mosquitoes, were carriers, the disease would spread more freely under certain circumstances than it was known to do. Later, direct experiment with both bed-bugs and fleas by Ricketts and Wilder, &c. (Rocha-Lima, 1919) has shown that neither of these insects conveys the infection by biting, nor after feeding contains an infective dose of virus. Persons who become infected with typhus are not necessarily 'lousy'. A single louse, which is sufficient to convey the infection, may be acquired by passing contact and may then be again lost in changed clothes; moreover, the irritation of the bite may be too trifling to attract notice.

Nicolle, Comte and Conseil (1909) found that body-lice which had sucked the blood of a chimpanzee, infected by the inoculation of blood from man, transmitted the disease to two monkeys on which they were subsequently fed for six days. At first the attempts to repeat these experiments by Ricketts and Wilder, Nicolle and Conseil, and Goldberger and Anderson gave rather doubtful results.

Rocha-Lima (1919) and Hegler and Prowazek (1914) infected guinea-pigs by inoculating them with part of an emulsion of a single louse. They estimated that the content of one louse was about 0·9 to 0·3 mgm. of blood, and since they found that it required 3 or 4 c.cm. of human blood to infect a guinea-pig they concluded that the multiplication of the virus in the louse must have been of the order of 10,000 times. These and similar experiments have indicated the very much greater concentration of the virus in the louse some days after its first infecting meal than in the patient's blood. Landsteiner (1915) and Rocha-Lima (1916^2) obtained similar results by intraperitoneal, subcutaneous or intracardiac inoculation of the organs of infected lice. Weil and Breinl (1924) found a hundredth part of an infected louse sufficient to infect a guinea-pig.

Arkwright, Bacot and Duncan (1919) infected a monkey in London, where there was no typhus, by subcutaneous and intramuscular inoculation with an emulsion of lice fed on typhus patients in Ireland, and from this monkey passed the disease to two other monkeys and to guinea-pigs by means of blood and lice. Wolbach, Todd and Palfrey (1922) in Poland, successfully transmitted typhus to guinea-pigs by inoculating them intraperitoneally with the viscera of infected lice, and they confirmed the infection by finding microscopic lesions in the brain and by the subsequent immunity of the guinea-pig or by passage of the disease to another animal.

The means by which lice transmit typhus. It has generally been assumed that lice infect man and monkeys by their bite. Cantacuzène (1919) gave instances of medical men who believed themselves to have been infected by the bites of one or at most two lice. Atkin and Bacot (1922) failed to obtain infection of a guinea-pig or a monkey by causing heavily infected lice to feed on these animals, taking care that the infective excreta of the lice did not remain on the skin or enter wounds. Nicolle, Blanc and Conseil (1916) and Nicolle (1922) showed that the excreta of lice infected with typhus virus were infective, and Arkwright and Bacot (1923) obtained five successful inoculations of guinea-pigs with louse excreta not more than 24 hours old. It seems probable that accidental infections of laboratory workers have often been due to the excreta of lice. Sparrow and Lumbroso (1929) succeeded in infecting guinea-pigs with virus (brain emulsion) dropped into the nares, and occasionally by application to the conjunctiva. Weil and Breinl (1924), by injecting separately the anterior parts of lice excluding the mid-gut but containing the salivary glands, obtained scarcely any evidence of infection in rabbits.

Exclusive transmission by lice. Observations and some experiments are on record which appear to prove that infection from typhus patients to a susceptible person does not occur in the absence of lice even when the healthy are in very close contact with the sick as bedfellows (Wilder, 1911; Jürgens, 1915).

The overwhelming experience during the war from many sides has made the 'louse-theory' of transmission to be universally accepted and has shown that at any rate under the conditions hitherto studied *epidemic* typhus is dependent on lice for its propagation. The means by which typhus, or typhus-like disease, is transmitted when lice are absent requires further investigation.

Persistence of the Virus between or apart from Epidemics.

How the virus of typhus is enabled to persist between epidemics is not understood. The hereditary transmission in the louse is not very probable and there is only very slight direct evidence in its favour (Rocha-Lima, 1919; Weigl, 1924). It is known that in endemic areas, where epidemics recur, many very mild, scarcely recognizable, attacks occur especially in children and these are believed to form a reservoir of infection. Weigl believes that complete immunity from typhus is not very long lasting and that second very mild attacks with short febrile periods of only 3 or 4 days' duration are common. There is the further possibility of an alternative mammalian host and insect or arthropod vector, but there is no direct evidence in favour of these latter hypotheses.

Whether the cases of 'sporadic typhus' in the U.S.A. and other countries are really instances of such means of transmission is not yet finally settled. Maxcy, Fletcher and Megaw all suspect ticks as the agents of infection in the United States, Malaya, and India respectively, and rats and mice have often been regarded as probable sources of the virus.

Lice.

Life-history, habits, &c.

The morphology, habits and life-history of human lice and their relation to disease have been studied by Hase (1915, 1919), Bacot (1917) and by Nuttall (1917) who also reviewed the literature on the subject. Lice are wingless parasitic insects which remain intimately associated with their vertebrate hosts throughout their life. They are all closely adapted to their habitat and are not known to live on more than one kind of host. Lice are oviparous and undergo an incomplete metamorphosis. There are two main divisions of the family; the *Anoplura* or blood-sucking lice, to which the human species belong; these feed exclusively on blood. The *Mallophaga* or biting lice constitute the large second division, which includes the common lice of guinea-pigs and birds, &c., which feed on epithelial debris, feathers, secretions of the skin, &c.

There are three kinds of human lice (1) *Phthirus pubis*, the crab-louse, which is not known to take any part in the transmission of typhus; (2) *Pediculus corporis* and (3) *Pediculus capitis*. The two latter are very much alike and are known to hybridize. They are often included in one species, *Pediculus humanus*. Morphologically there are only slight differences. The head-louse is on the average slightly smaller and narrower in the body and has the lateral margins of the abdomen more deeply indented. The eggs of *Pediculi* are oval in shape and about 1 mm. long. They are firmly cemented to hairs or threads on clothing and are very difficult to remove. They hatch in 4 to 8 days in a temperature of 35 to 36° C. Below 22° C. hatching does not occur, but the eggs may remain viable 25 days (Bacot) or rarely 5 weeks (Nuttall). The larvæ when first hatched are very small and active. There are three larval stages and three moults before the adult stage is reached. Each stage lasts about 3 or 4 days, and the total period from the time the egg is hatched till the adult emerges is 8 to 10 days under optimal conditions, but may be prolonged to 16 or 17 days. The larvæ and adults suck blood several times daily. The minimum time required for the complete cycle of *P. corporis* from egg to egg under good conditions is about 16 days. An adult louse has been kept alive 46 days. It is estimated that a single female may lay 300 eggs and might have 1,918 descendants in her lifetime.

Probably on discarded clothes kept at 15° C. (59° F.) egg-laying ceases. The male louse is slightly smaller than the female and may be recognized by the V-shaped copulatory apparatus which is on the ventral surface of the abdomen when at rest. The mouth apparatus consists of pricking stylets and hooks sunk in a pit which is everted in the act of sucking (Peacock, 1918). There are two pairs of salivary glands whose secretions no doubt facilitate feeding by causing congestion and by inhibiting the clotting of the blood, and also account for the resulting wheal or papule. The alimentary canal is short. The narrow gullet opens into the bag-like mid-gut, which is succeeded by the hind-gut with blind malpighian tubules attached and a short rectum with six glandular folds. No regurgitation has been observed from the midgut to the gullet. The fæces are passed out as solid brown granules.

Disinfestation.

Lice and their eggs are both easily killed by heat. A temperature of 52° C. for half an hour is sufficient under laboratory conditions (Bacot. 1916; Bacot and Talbot, 1919). A chamber containing hot air at 55° C, will disinfest clothes in 30 minutes if they are hung up separately. If steam is used the temperature of the chamber should be kept at not less than 65° C. Usually failure to kill lice or their eggs by heat is due to clothes or bedding being rolled up in bundles or put in heaps so that the heat does not penetrate and the temperature in the interior of the bundle is not much raised. For bundles of clothes a chamber with a temperature of 100° C. for 2 or 3 hours at least is needed (Bacot, 1917[1]; Grant and Bacot, 1918).

Immersion of bedding or clothing in water kept at 65° C. for 30 minutes destroys all nits and lice. Soaking in 2·5 per cent. lysol for 20 minutes is sufficient without heat if the articles are afterwards dried without rinsing. Fumigation has generally proved unreliable. In order to destroy lice and eggs in cloth garments a hot flat-iron is very useful.

To free the hairy scalp from lice and eggs insecticides are necessary (Bacot, 1921; Bacot and Lloyd, 1919). The best applications are composed mainly of grease or oil. If lice are immersed in greasy material for 5 to 20 minutes they are killed. One part of oil of sassafras, tar oil or light-wood oil added to 20 parts of kerosene or paraffin oil very much increases the efficacy of this excipient, especially in the case of the eggs. Several other essential oils are known to be similarly insecticidal. As it is difficult in practice to ensure complete saturation of the hair the application of the oil should be followed by enveloping the hairy surface with some impermeable covering for an hour or more. Several applications to the scalp are in practice found necessary, or at least two with an interval of ten days, to ensure killing lice hatched from eggs that have survived the first application. Where lice are common it is well to keep the hair close cropped to avoid infestation, but shaving is inadvisable in epidemics since the slight wounds inflicted may assist the entry of virus.

Relative importance of P. corporis *and* capitis.

The most important differences between *P. corporis* and *capitis* are in regard to their habits. *P. capitis* lives in the hairy scalp, which it does not leave at all during its life. The eggs are cemented to the hairs and unless these are cut or shed the eggs, as well as all stages of the insect, as a rule, remain on the human body. The head-louse is accustomed to cooler surroundings than the body-louse, and it is perhaps partly because the hairy scalp is a cooler situation than the skin of the body under clothes that *P. capitis* is found in warmer climates than *P. corporis*. *P. corporis* frequents all parts of the body covered by clothes and in cases of gross infestation the outer clothes as well as those next the skin are chosen for the deposit of eggs or nits. It shelters in the clothes when not actually feeding and passes into the outer layers of clothing especially under conditions of high temperature or when sweating is profuse. When the clothes are taken off at night some lice are often shaken on to the ground and in other ways come in contact with new hosts. Since the lice prefer a temperature approximating 30 to 36° C., they attach themselves readily to passing human beings in preference to the chilled garments put aside at night or discarded by the sick or those who have died of typhus. The bedding of patients also becomes infested. Lice may remain alive under cool conditions without feeding for some days, and eggs attached to clothing, if kept in the cool, often remain viable for over a fortnight.

The contrast between the habits of *P. corporis* and *capitis* is, therefore, very marked in relation to their ability to spread diseases. *P. capitis* does not pass so readily from one host to another. However, when infestation with *P. capitis* has occurred the parasites are more persistent in spite of washing and care, and are more difficult to kill, since disinfectants used must not be strong enough to injure the host, and heat for the same reason is inapplicable. *P. corporis* is relatively easy to get rid of; even a thorough weekly wash of clothes in hot water interrupts the life-cycle. When day clothes are taken off at night the lower temperature checks the reproduction of the lice.

Experiments in the laboratory have shown that the virus of typhus can develop in the head-louse if kept at 30 to 36° C., and be used for transmission of the infection (Goldberger and Anderson, 1912; Rocha-Lima, 1919).

The distribution of typhus fever, especially in its epidemic form, is strongly in favour of *P. corporis* being the usual vector. For instance, the epidemic disease is not found in countries or districts where *P. corporis* is absent on account of the heat of the climate or for other reasons.

Experiments with Lice.

In order to carry out satisfactory experiments with *Pediculi* it is important to use a healthy stock of lice which has been reared in captivity from eggs obtained in a place free from louse-borne diseases. The lice should have been fed on healthy persons for one or two generations and examined in the meanwhile for any evidence of infection. Several kinds of boxes have been used by different workers in which to keep the lice securely confined, but so contrived as to enable them to feed without escaping and to live at a suitable temperature. The boxes may be made of wood, cardboard or metal, but in the latter case the surface is liable to become wet from condensation ; it is very important that they should be very strong lest they become broken and infected lice escape. Strong glass-topped entomological pill-boxes are suitable for the purpose. Some coils of thread or wool should be placed inside to give foothold to the lice and to allow for attachment of the eggs.

The opening of the box has a covering of fine gauze affixed with thread and gum. Silk 'bolting cloth' with a mesh of 70 to the inch is strong and the best for the purpose. This prevents even the smallest young larvæ from escaping but enables all the lice to suck blood through it. When the lice are fed the lid of the box is removed and the gauze-covered opening firmly applied to the bare skin and kept in place with bandages or straps for half an hour or more at a time, two or three times a day. Wolbach, Todd and Palfrey (1922) describe a suitable metal form of box. Infected lice must only be fed on an immune person for fear of communicating infection. Between the feeds the boxes must be kept in an incubator at a temperature of 33 to 35° C. (91·8 to 95° F.), which also contains a small vessel of water to prevent undue dryness.

The handling of infected lice is a very dangerous procedure and a very high proportion of research workers thus engaged have become infected, and not a few have died in consequence. Amongst the most distinguished who have lost their lives as a result of their researches may be mentioned Ricketts, v. Prowazek, Jochmann, Schüssler, Bacot, Weil and Cragg. It is, therefore, important that those dealing with infective lice or the boxes containing them should use every precaution to prevent the escape of lice. Care must be taken also to avoid wounding the skin, and any scratch or abrasion must be protected. Also, infected particles of excreta must not be conveyed accidentally to the conjunctiva or air passages. The wearing of rubber gloves and spectacles is very desirable, and the infective excreta must be prevented from contaminating surrounding objects. The excreta can be collected in the form of a fine brown powder through the gauze if the box is held over a receptacle and lightly tapped.

Artificial Feeding.

No practicable method has yet been devised by which lice can be made to feed by the mouth otherwise than by sucking blood from the skin of man or monkey. They do not feed readily on monkeys and there is a high mortality amongst lice fed on these animals. If lice are hungry they will suck the blood of a guinea-pig, but they invariably die in the course of a few hours as they appear to be unable to digest this blood.

Weigl's technique of anal feeding. A method was devised by Weigl (1920) and practised extensively by himself and his colleagues and by Bacot (1922) for feeding lice artificially. The lice are given whipped human blood through the anus with an exceedingly fine pipette. In this way lice have often been kept alive, in apparent health and able to lay eggs for 7 to 10 days. Unless the outside of the louse is carefully disinfected, artificially fed lice often die from bacterial infection of the intestine (Bacot, 1922)

Conditions of the Infection of Lice in Captivity.

If lice are fed on an infected man for 1 or 2 days after the fourth day from the onset of fever, and are subsequently kept alive and healthy by regular feeding for 7 to 10 days on the same patient, or on an immune person, the lice become infective and usually remain so for life. The facts learnt from experiments made with lice of a known healthy stock confined in boxes have confirmed and made more exact the knowledge gained from lice captured on patients (Rocha-Lima, 1919 ; Jungmann and Kuczynski, 1917 ; and Wolbach, Todd and Palfrey, 1922). Since lice cannot be kept alive after feeding naturally on the guinea-pig, Weigl (1920) injected them *per anum* with a very minute quantity of washed leucocytes from infective guinea-pigs' blood and the lice were subsequently fed with whipped human blood or allowed to feed on a monkey or immune human being (Weigl, 1920, 1924[1 & 2]; Bacot and Ségal, 1922). The experiments made in this way by Weigl and Bacot have shown that the virus after being passed from man to the monkey or guinea-pig by inoculation of infected blood or lice may then be passed from the infected monkey or guinea-pig again to lice by inoculating these insects *per anum*, and after incubation in the louse returned again to a mammalian host. In this way the reality of the infection of the monkey and guinea-pig with typhus has been demonstrated and the identity of the virus in the mammalian and insect hosts has been confirmed. Weigl and Bacot also carried infection from louse to louse in this way.

The 'Genus' Rickettsia.

This name has been used generically for certain minute bodies which are found in the gut of insects. It was first applied by Rocha-Lima to the form *R. prowazeki*, observed in lice infected with typhus virus. He proposed the name in honour of Ricketts, who first called attention to these bodies, and Prowazek, who further studied them. The name is also used for other very similar bodies, *R. quintana*, found in lice taken from trench-fever patients, and the species present in ticks infected with the virus of Rocky Mountain fever. The resemblances between these three species of Rickettsia is very striking. Each of them is associated with a definite disease in man and is found in enormous numbers in the arthropod host. In each species the forms of rickettsia are mainly very small, 0·5μ or less in diameter. They are non-motile, Gram-negative and stain feebly with aniline dyes, but can be coloured by prolonged use of weak

Giemsa stain. *R. quintana* is found free in the lumen of the gut, *R. prowazeki* in the lining cells and the lumen of the midgut of *Pediculi* and the rickettsia of Rocky Mountain fever (*Dermacentroxenus rickettsi* Wolbach) is present in the cells and lumen of the gut and in other parts of the infected tick. The use of the name *Rickettsia* has been extended to include minute forms found in many parasitic insects and arachnids (see Cowdry, 1923). Some of these are not known to be pathogenic. *R. melophagi* (Nöller, 1917) occurs in immense numbers on the surface of the epithelium lining the gut of the sheep-ked, a pupiparous wingless fly, *Melophagus ovinus*. It is always present and apparently hereditary and variable in morphology in the ked (Arkwright and Bacot, 1921; Anigstein, 1927). Though no pathological condition of the sheep associated with this rickettsia is known, presumably the microbe is present in the sheep's blood since it has been obtained in cultures from this source by Nöller and Kuchling (1923).

R. lectularii is a constant inhabitant of the bed-bug (*Cimex lectularius*) and multiplies in the epithelial cells of the Malpighian tubules, whence it escapes into the gut. Like *R. prowazeki* it grows inside cells in long threads as well as in minute ovals and rods, which constitute the bulk of the micro-organisms found closely packed in the infected cells.

Cowdry (1925) has described a micro-organism, *R. ruminantium*, as the cause of heartwater, a disease of sheep. The vector of this microscopic parasite is a tick (*Amblyomma hebræum*). In this case the rickettsia is found chiefly in the cells of the kidney of the sheep. It is also described in the tick, but is not distinguished there so readily.

Cowdry collected the records and described a large number of bodies in insects and other arthopods which he regarded as similar to rickettsia, and suitably placed in the same class of microbes. Hertig and Wolbach (1924) catalogued some 45 species in different insects. Most of these latter are blood-suckers or feed on animal tissues, but this is not always the case, since some of these rickettsiæ inhabit arthropods which are not parasitic and live on quite different kinds of food, e.g. rickettsia of *Psokus* (Sikora), the dust- or book-louse, and of *Lepsima*, known as 'silver fish'.

The class rickettsia is exceedingly difficult to define. Hitherto the use of the name has not been restricted even to parasitic blood-sucking insects. Hertig and Wolbach (1925) suggested reserving the name for 'proved pathogenic micro-organisms of very small size, pleomorphism, slight affinity for aniline dyes and intracellular habitat'. *R. quintana*, which is associated with trench fever, however, is not found inside cells and varies but little in its morphology. If the work on *R. melophagi* by Nöller and Kuchling (1923) is accepted, cultures of this rickettsia can be obtained from the blood of the sheep, which would seem to prove that the rickettsia occurs in this animal although there are no recognized symptoms of disease.

R. lectularii is believed to be hereditary in *Cimex* and is not known to be present in the blood of any mammalian host of the bed-bug.

The occurrence of heredity of a rickettsia in the arthopod, though very important as regards the transmission and epidemiology of disease, is perhaps as much dependent on the kind of insect or tick as on the special peculiarities of the microbe. None of the species of rickettsia in the louse have been proved definitely to be hereditary, but those found in the ked and the bed-bug and some species in ticks are considered to be so.

It seems doubtful whether the various species of *Rickettsia* described are nearly related. Their resemblançe is perhaps partly due to their parasitism of the invertebrate hosts. There is no doubt that rickettsia for the most part closely resembles a small bacterium, and this is especially the case with a species like *R. prowazeki* which grows as threads or chains, and it has been suggested that this species is a stage in the life-history of a bacterium such as *B. proteus X19*, which is otherwise closely connected with typhus fever.

It seems best that the name ' rickettsia ' should only be applied when the micro-organism consists chiefly of very minute forms found in the intestine of a blood-sucking arthropod, and when there is good reason to suppose that the rickettsia is derived from the host's blood. The name should only be further extended to similar microbes when the resemblance to a well-characterized species is very close.

Great difficulties have been encountered in attempts at artificial cultivation of the pathogenic species of rickettsia, but *R. melophagi* has been grown from the sheep-ked in colonies on agar containing heated blood by Nöller (1917) and Jungmann (1918) and from sheep's blood by Nöller and Kuchling (1923), Anigstein (1927) and Hertig and Wolbach (1924). Sikora (1921) reported having obtained cultures of *R. pediculi* from *Pediculus corporis* on blood-agar. Several workers have also made not very well substantiated claims to the cultivation of *R. prowazeki.*

Rickettsia prowazeki.

Rickettsia prowazeki was first recognized by Ricketts and Wilder (1910) in lice from typhus-fever patients. These writers, unlike most subsequent workers, believed that they could also identify the same bodies in films of the blood from patients.

Description of R. prowazeki *and its occurrence.*

Sergent, Foley and Vialatte (1914, 1921) described thousands of minute oval bodies in stained smears of the contents of lice from human patients. Prowazek and Rocha-Lima in Poland in 1914–15 rediscovered these bodies in infected lice and Prowazek also thought he could detect them in human blood. After Prowazek's death the work was continued by Rocha-Lima, by whom the main facts about the infection of lice with rickettsia were established. He summarized the knowledge of the *R. prowazeki* and typhus virus in 1919. *R. prowazeki* is best stained with slightly alkaline weak Giemsa for 10 to 20 hours and takes a violet or reddish tint. It varies considerably in morphology. Most commonly the forms are round or oval, $0{\cdot}2 \times 0{\cdot}2$ to $0{\cdot}2 \times 0{\cdot}3\mu$, or they may be slightly rod-shaped,

$0{\cdot}2 \times 0{\cdot}5\mu$. They may appear as two dots with an unstained interspace or as dumb-bells. In other specimens which perhaps correspond to an early stage of louse infection (Wolbach, Todd and Palfrey, 1922) they occur in threads or chains of 2 or 3 or even 10μ long (Otto and Dietrich, 1917 ; Arkwright and Bacot, 1923[1]). Typically they are found inside the epithelial cells lining the midgut of the louse where they grow and multiply, filling the cell, and whence they are discharged into the lumen of the gut by rupture of the cells and are passed out in the fæces. The intracellular habitat of *R. prowazeki* was first described by Rocha-Lima and is the most certain criterion by which it can be distinguished from *R. pediculi* or *R. quintana*, which were very common in lice in Europe during the war.

R. prowazeki may also be seen in large numbers in louse fæces. *Rickettsia prowazeki* was studied in very thin serial sections of the gut of the louse by Rocha-Lima, Wolbach, Bacot and others, and also in smears of the walls of the gut and the gut contents. The former method is no doubt superior as a means of deciding whether the bodies are intracellular, but this position can often be well demonstrated in films.

Identity of R. prowazeki *and the virus of typhus.*

Rocha-Lima (1916–19) made a careful study of the conditions governing the appearance of rickettsia in lice and the association of these bodies with the virus. He maintained that rickettsia was a living microbe which was present in the blood but was unrecognizable microscopically there, probably on account of its lack of distinctive appearance and the relatively small numbers present. Lice confined in boxes were fed on patients at different stages of the disease and for varying periods. Samples of these lice were then examined by microscopic sections, and it was found that the *R. prowazeki* occupied and multiplied in cells of the midgut, but that the other kinds of rickettsia which were very commonly found in lice during the war, i.e. *R. pediculi* and *R. quintana*, were only found in the lumen and not inside cells. One species, *R. rocha-limæ*, described by Weigl (1921) in *P. corporis*, like *R. prowazeki*, also grows in threads and enters the cells of the louse gut. Its occurrence has only certainly been recorded by Weigl, who says that it is distinguishable from the other species by its morphological peculiarities and variability. Arkwright and Bacot (1923) met with a somewhat similar rickettsia in a small series of lice in Cairo, but it did not quite answer to Weigl's description of *R. rocha-limæ*, and he calls it *R. cairo*.

The presence of typhus virus in a louse can usually be detected by examining a film of the midgut and its contents for *R. prowazeki*. The virus and the rickettsia are so constantly associated that it is an almost inevitable assumption that the virus is identical with the rickettsia. A louse which contains numerous *R. prowazeki* has always been found infective unless the louse has been dead some time, or both the virus and rickettsia have been killed as the result of treatment by heat or drying, or perhaps sometimes by the action of bacteria in the louse. *R. prowazeki*, like the virus, is not found in lice unless sufficient time has elapsed since they were first fed on infected blood and they have been kept at a suitable temperature in the meanwhile.

It is essential that lice confined in boxes should be kept at a temperature of about 30 to 35° C., since if the temperature is lower *R. prowazeki* does not multiply freely and if the temperature is only 23° C. no development takes place.

If *P. corporis* are fed on a febrile patient between the fourth and eighth day of the disease and are subsequently fed for 7 to 10 days on the same man or a convalescent or recovered patient, *R. prowazeki* is found in great profusion in the gut of the lice. The number of rickettsia found in the lice increases with time after the first infecting feed, and the proportion of lice infected in a box also increases with the number of days on which they have sucked infective blood. Rocha-Lima found 95 per cent. of lice infected with *R. prowazeki* if they were taken from a patient at an advanced stage of the illness. The development of infectivity of lice with lapse of time after they have fed on a patient is probably merely the result of the increase in the numbers of the rickettsia, and not of the appearance of any different stage in the life-history of the infective agent.

It is seen then that the conditions of feeding and keeping the lice required to produce a recognizable infection either with typhus virus or rickettsia are the same.

Carefully observed instances of the simultaneous appearance in lice of virus and rickettsia have been recorded by Wolbach, Todd and Palfrey. In a series of experiments in which 41 lice were separately examined from 10 boxes fed on typhus patients it was found that none of the lice were infective from boxes in which no *R. prowazeki* could be found, and that every louse known to contain rickettsia was infective. Four lice were, however, infective in which rickettsia were not found.

The authors of these experiments remark that they found it more difficult to infect lice with *R. prowazeki* than Rocha-Lima had implied, and they always found some lice in a box uninfected, but the proportion which became infected with *R. prowazeki* was of much the same order as that which became infected with virus.

Wolbach, Todd and Palfrey (1922) found *R. prowazeki* in those louse fæces which were infective. It is probable that the virus is dead when rickettsia are seen but the fæces do not infect. Arkwright, Bacot and Duncan (1919), Atkin and Bacot (1922) and Arkwright and Bacot (1923²), in small series of experiments found the same association of virus and rickettsia in lice. Weigl (1920) and Bacot and Ségal (1922) in addition demonstrated *R. prowazeki* and virus in lice which had been inoculated *per anum* with the blood of a monkey or guinea-pig taken during the infective stage of the fever. By this means they obtained direct evidence of the presence of *R. prowazeki* or its precursor in the blood of the typhus monkey and guinea-pig.

Rickettsia in P. capitis.

It has been shown by Rocha-Lima and Sikora that *P. capitis*, after an infective feed, if kept at a suitable temperature, contains *R. prowazeki*, like *P. corporis*, and Bacot confirmed this observation. It is, however, generally accepted that *P. capitis* is of comparatively little importance in epidemic typhus.

It has been mentioned above that in the sporadic typhus of New York and the South-Eastern United States, in the tropical typhus of Malaya and in some parts of India, *Pediculi* are usually said to be completely absent from the patients and their surroundings, but in some cases the presence of head-lice has been mentioned. It seems certain that if the spread of infection were due to *P. capitis* in any group of cases the rate and ease with which the virus was distributed would be very different to that seen in epidemics associated with body-lice. There have been no recorded experiments in which lice have been fed directly on patients with Brill's disease of the United States or on the similar cases in other parts of the world. Mooser and Dummer (1930[1 & 2]), however, report that they have infected *per anum P. corporis* with material from the testes of rats inoculated with both Mexican typhus and the sporadic typhus of North America, and that *Rickettsia prowazeki* developed in the louse, which could then be used to transmit the infection to guinea-pigs.

Criticisms directed against the importance of R. prowazeki *in typhus fever.*

At first the importance of rickettsia in disease was denied by Nicolle, Blanc and Conseil (1914), Brumpt (1918) and others on the ground that these bodies were found in lice taken from persons not affected with typhus. This opposition was probably the result of confusion of *R. prowazeki* and *R. quintana.* During the war trench fever was very prevalent and often unrecognized and *R. quintana* consequently very common in lice. The two species are often very much alike in louse fæces or in the intestinal contents, though they can be definitely differentiated by the presence of threads or chains or by the intracellular position of *R. prowazeki.* Other criticisms of the ætiological importance of rickettsia bodies have been made on the ground that they are not microbes but either cell granules or degeneration products of red blood corpuscles, nuclei or platelets (Woodcock, 1923, 1924) or of bacteria, &c. (Friedberger, 1916). Although, when very small and round and in small numbers, the recognition of rickettsia may be a matter of some difficulty, the staining reactions, range of morphology, occurrence in masses and especially the regularity of appearance in relation to the stage of the disease, the time of feeding, &c., quite negative such hypotheses. The differentiation of rickettsia from other granules by its staining properties has been especially dealt with by Nicholson (1923) and Cowdry and Olitsky (1922).

Occurrence of R. prowazeki *in* Pedicinus longiceps.

Macacus rhesus is often the host of a small blood-sucking louse, *Pedicinus longiceps*, which multiplies rapidly on sick monkeys. Arkwright, Bacot and Duncan (1919) examined films of the alimentary canal of 93 of these lice collected from three macaques infected with typhus from the seventh to the seventeenth day from the onset of fever. Of these, 12 (13·2 per cent.) contained numerous minute oval bodies like rickettsia, whereas in 75 lice of the same species from three healthy monkeys no rickettsia was found. The rickettsia from the *Pedicinus* was rather smaller than *R. prowazeki* is usually. Atkin and Bacot (1922) made an emulsion of the gut of 36 of these lice from an infected monkey and found numerous rickettsiæ in the emulsion. This emulsion was then injected into a healthy monkey, which, as a result, had a typical attack of typhus-like fever and subsequently proved resistant to a large dose of virus. In all, 150 *Pedicini* from healthy monkeys have been examined and all have been free from rickettsia.

Culture of R. prowazeki.

Attempts to propagate *R. prowazeki* on artificial media have failed in the hands of very many observers. Successful cultivation *in vitro,* however, has been claimed, but it has not usually been maintained that *R. prowazeki* has multiplied in the form in which it occurs in the louse. Kuczynski (1927) summarized his long series of elaborate investigations and claimed to have cultured micro-organisms of variable morphology but often closely similar to *Rickettsia prowazeki.* He was successful in a high percentage of cultures made with a variety of typhus-infected materials from human and animal sources. He tried many kinds of culture media but was most successful with those containing partially digested animal proteins and ascitic fluid heated at 60° C. He also described forms intermediate between rickettsia and *B. proteus* and believes both these organisms to be stages in the life-history of *B. proteus X19.* These observations have not been generally accepted as yet. Weigl (1923), Fejgin (1927^2) and Anigstein and Amzel (1927) claim that they obtained growth *in vitro* of rickettsia of variable morphology, and the two last workers further (1930) report a long series of experiments confirming Kuczynski.

R. prowazeki *in the tissues of the mammalian host.*

The presence of *R. prowazeki* in endothelial cells of minute arterioles in man has been described by Kuczynski and Jaffé (1919) and also by Wolbach, Todd and Palfrey (1922). The cells containing the bodies believed to be rickettsia were few and the evidence that they were actually this micro-organism has not been universally accepted. The probability of such an intracellular location of *Rickettsia prowazeki* in mammals has been, however, considerably enhanced by the more regular demonstration of the rickettsia of Rocky Mountain fever in endothelial cells of the testis of infected animals. It has further been demonstrated by Wolbach and Schlesinger (1923) that both the *Dermocentroxenus* of Rocky Mountain fever and the rickettsia of typhus multiply in tissue cultures. In the case of *R. prowazeki* the tissues cultured were from the brain of infected animals and the site of propagation the endothelial lining of the minute vessels. Moreover, Pinkerton (1929), Maxcy (1929[1 & 2]) and Mooser (1930[1 & 2]) describe the occurrence of forms, often within cells, closely resembling *R. prowazeki,* in the exudate on the surface of the testis in guinea-pigs and rats, when the virus inoculated is from the typhus of Mexico, the South-Eastern States or Europe. Cowdry (1925) described *R. ruminantium* both in the tick vector and also inside endothelial cells of the sheep infected with ‘ heartwater ’.

Differential diagnosis of the different species of Rickettsia.

The differential diagnosis of the different species of *Rickettsia* in human pediculi and their relation to the lice has been surveyed by Weigl (1924[2]). It may be added that *R. pediculi* as described by Rocha-Lima is probably identical with *R. quintana,* and that *R. prowazeki* appears to be only present in the blood during the febrile stage of the mammalian disease, whereas lice can become infected with *R. quintana* by feeding on trench fever patients who are afebrile and have even had no recognizable symptoms for weeks or months.

Properties of the Virus of Typhus Fever.

Filtration. Attempts to obtain active virus in filtrates from efficient bacterial filters have usually been completely unsuccessful. The experiments by Goldberger and Anderson (1912), Prowazek and Rocha-Lima and others, are summarized by Rocha-Lima (1919) and give no support to the earlier claims of filterability made by Nicolle. More recently Fejgin (1927[2]) has stated that she can obtain active filtrates of certain stages of the virus. Hach (1926) claimed to be able to transmit the disease to guinea-pigs with very large doses of a filtrate from the organs of infected animals.

Resistance of the virus. The virus has very little capacity for resisting adverse physical or chemical agencies. Infective blood kept for 2 days at 20 or 37° C. has proved inactive and a temperature of 55° C. has always been found to destroy its activity in 15 to 30 minutes even when the virus is in the very concentrated form in which it is found in the contents of the louse. Blood kept at 0° C. for 8 days has proved infective, but only a few observations on this method of preserving virus have been recorded (Goldberger and Anderson, 1912). Blood dried in a desiccator over sulphuric acid had lost its activity. Nicolle, Blanc and Conseil (1914) found that excreta of lice dried in the air lost their virulence in a few days. Phenol 0·5 per cent. and the addition of glycerol has been found to inactivate virus.

PREVENTIVE MEASURES.

By far the most important means of prevention are those required to free the patients and the rest of the population from lice, including careful handling of verminous patients and their clothes, and louse-proof